The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 16 MARCH, 1950 NUMBER 2

# THE ISOLATION AND IDENTIFICATION OF PATHOGENIC FUNGI*

CHARLOTTE C. CAMPBELL

*From the Department of Bacteriology, Army Medical Department Research and Graduate School, Army Medical Center, Washington 12, D. C.*

Until recently it was the general belief of the medical profession that mycoses other than those of common ringworm were rare. Furthermore, since very few individuals interested themselves in the field, opportunities for training available to those who desired it were limited. The now well-defined endemic areas of histoplasmosis and coccidioidomycosis, the relatively frequent isolation of *Actinomyces bovis, Blastomyces dermatitidis* and *Cryptococcus neoformans,* the recurrent outbreaks of tinea capitis among school children as well as the tell-tale cracks of "Athlete's Foot" between one's own toes suggests that the pathogenic fungi are being encountered as often as such bacterial pathogens as *Corynebacterium diphtheriae* or *Salmonella typhi.* The medical technician who could not identify the latter species would be considered ill-prepared indeed and yet the same technician is quickly forgiven if he fails to recognize the more common fungus pathogens. It is time to realize that shortcomings in this field can and should be overcome.

It cannot be implied that medical mycology can be mastered without appropriate effort on the part of those interested in learning. On the other hand, the subject can no longer be considered so specialized that useful and practical knowledge cannot be obtained in a relatively short time. The purpose of this paper is to assure the medical technician that time and experi-

* Presented before Convention, Roanoke, Va., June, 1949.

ence spent in learning to isolate and identify the medically important fungi will greatly augment his utility in the diagnostic laboratory.

The following general outline is presented as a basic practical classification to which additional information can be added as time and opportunity permit. It is meant to be a guide, not a complete work.

Through the extensive studies of Conant, Martin, et al,[1] Lewis and Hopper[2] and other contemporary workers,[3,4] the species of fungi known to be pathogenic for man have been definitely established. Through these same workers, nomenclature, synonomy and terminology, always obstacles to persons not botanically trained, have also been simplified. Elucidation of the known characteristics of fungus pathogens is analogous in every way to knowledge of the bacterial organisms. One is cognizant of the species essential to the preparation of certain foods and the important role of others in industry and agriculture, but in the medical laboratory the primary interest is directed toward those species capable of producing disease in man.

From the practical viewpoint it is necessary to know only a few fundamentals. Whereas bacterial species reproduce by binary fission, many fungi are multicellular and reproduce by spores which may enlarge and send out tube-like processes (germ tubes). These elongate at the distal end becoming long filaments that eventually branch. Each filament is a hypha (pl. hyphae) which may or may not become divided into a chain of cells by the formation of transverse walls. Filaments which form such walls are called septate hyphae, those which do not are non-septate (coenocytic). A mat of growth of massed hyphae is called a mycelium.

The cottony, filamentous colonies usually produced by such fungi are known as "molds." Other fungi are unicellular organisms that reproduce by simple budding and are commonly referred to as "yeasts." Yeast colonies are similar in appearance and consistency to those produced by many bacterial species. As in Candida species the colonies are sometimes composed of single-celled budding forms and broad hyphal elements in combination.

All pathogenic fungi, except Actinomyces and Nocardia species belong to the class Fungi Imperfecti in which no sexual cycle of reproduction has been demonstrated. Actinomyces and Nocardia species are classified with the bacterial species as Schizomycetes. The multicellular fungus pathogens have septate hyphae from or in which specialized spores, characteristic as to size, shape and mode of development for each species, are produced. They also grow much more slowly than bacterial species or saprophytic fungus contaminants.

Neophytes in the field, therefore, learn to recognize the specialized spore characteristics of each pathogenic species and the type of colony from which it is produced, whether yeasty (of bacterial consistency) or moldy (filamentous, cottony). Biochemical reactions such as carbohydrate fermentative patterns (except in the genus Candida), antigenic analysis and other bacteriological procedures are, in their present experimental status, of little value in identifying the mycotic pathogens. Skin and serological tests with various fungus antigens may assist the clinician in his evaluation of a particular clinical syndrome, *but absolute diagnosis of any mycotic disease can be established only through the isolation and identification of the causative agent.*

Classification is begun by dividing the pathogenic species into two large categories according to the clinical manifestation of the disease produced.

I. *The dermatomycoses and other superficial mycoses* are diseases in which only hair, skin and nails are attacked. The agents of these mycoses do not penetrate the deeper tissues and are not potentially fatal. (Table I).

II. *The subcutaneous and the systemic mycoses* are caused by fungi which may penetrate the deeper tissues, spread by way of blood and lymph, and eventually invade many of the major organs. (Table I).

**TABLE I**

**Practical Classification of Pathogenic Fungi**

| DERMATOMYCOSES AND SUPERFICIAL MYCOSES | SUBCUTANEOUS AND SYSTEMIC MYCOSES |
|---|---|
| **Organisms found in hair, skin, nails** | **Organisms found in pus, sputum, spinal fluid, biopsy specimens, etc.** |
| **A. Dermatomycoses:**<br>1. *Microsporum* sp.<br>2. *Trichophyton* sp.<br>3. *Epidermophyton* sp.<br><br>**B. Superficial Mycoses:**<br>1. *Malassezia furfur* (tinea versicolor)<br>2. *Nocardia tenuis* (trichomycosis)<br>3. *Pedraia Hortai* (Black piedra)<br>4. *Trichosporon Beigelii* (White piedra)<br>5. *Nocardia minutissima* (erythrasma) | **A. Bacteria-like:**<br>1. *Actinomyces bovis* (actinomycosis)<br>2. *Nocardia asteroides* (nocardiosis)<br>3. *Nocardia madurae* ("Madura foot")<br><br>**B. Yeast-Like:**<br>1. *Cryptococcus neoformans* (cryptococcosis)<br>2. *Candida (Monilia) albicans* (moniliasis, thrush, vaginitis, etc.)<br><br>**C. Yeast-Mold:**<br>1. *Blastomyces dermatitidis* (North American blastomycosis)<br>2. *Blastomyces brasiliensis* (South American blastomycosis)<br>3. *Histoplasma capsulatum* (histoplasmosis)<br>4. *Sporotrichum schenckii* (sporotrichosis)<br><br>**D. Molds:**<br>1. *Coccidioides immitis* (coccidioidomycosis)<br>2. *H. compactum, H. pedrosoi, P. verrucosa* (chromoblastomycosis)<br>3. Monosporium, Aspergillus, etc. (maduromycosis) |

## I. Dermatomycoses and Other Superficial Mycoses

Dermatophytic species are agents of ringworm of the scalp, barber's itch, athlete's foot, etc. Their presence in infected material can often be detected by direct microscopic examination. Branched or unbranched hyphal filaments in nails or skin and small spores within or directly surrounding the hair shaft are sufficient evidence that the agent in question is a dermatophyte. The exact genus and species, however, can be determined only through isolation and identification of the organism. Three genera are involved.

1. *Microsporum.*—There are three species, *M. audouini, M. lanosum* and *M. gypseum.* These attack only hair and skin. Colonies vary with species and strain in the amount and type of aerial mycelium (cottony growth above the surface of the medium) produced, but primary isolates of all three organisms are readily identified microscopically by LARGE spindle-shaped, septate spores (macroconidia, fuseaux) (Fig. 1). Minor variations in the shape and size of the macroconidia serve to differentiate the species. In *M. lanosum* and *M. gypseum* the macroconidia appear in large numbers; in *M. audouini* they are rare and frequently for production require culture on honey agar containing 5 mg/ml of yeast extract.[5] Although a few small spores (microconidia) are produced in this genus, the macroconidium is the spore structure by which it is identified.

2. *Trichophyton.*—There are at least nine valid pathogenic species belonging to this genus. They may attack hair, skin or nails. The nine species are divided into four groups: (1) Gypseum, (2) Rubrum, (3) Crateriform and (4) Faviform. Fortunately, however, the two groups (also species) which are most commonly encountered *(T. mentagrophytes* and *T. rubrum)* are the exact microscopic counterpart of the genus Microsporum, in that microconidia (SMALL round or oval spores arranged in clusters or singly along the hyphae) are produced in abundance and the macroconidia (Fig. 2) are rarely seen. The latter, when present, are differently shaped and in other ways easily distinguishable from the macroconidia of Microsporum. The presence of numerous microconidia indicates the microscopic classification of these two species. Spiralling or coiling of the hyphae is also seen in many strains of *T. mentagrophytes.* A reddish-purple pigment produced at the back of the colony and diffusing throughout the medium further differentiates *T. rubrum* from *T. mentagrophytes* even without the subtle differences of the microscopic pattern. White aerial hyphae are produced by both species.

Organisms belonging to the Faviform group *(T. schoenleini, T. concentricum, T. ferrugineum* and *T. violaceum)* frequently require 3 to 4 weeks incubation periods for isolation. They develop waxy,

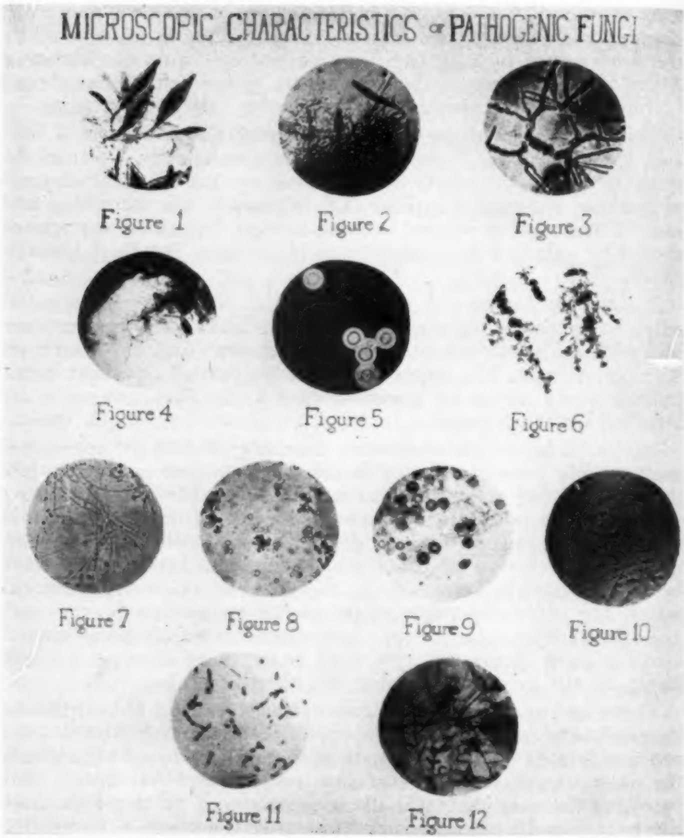
smooth, compact colonies, which vary in color (brown, purple, orange) with the species. Microscopically, they are identified by the presence of hyphal swellings known as "favic chandeliers." There are no characteristic spore forms. Suspected species should be referred to a competent mycologist for final identification.

The four species of the crateriforme group *(T. tonsurans, T. epilans, T. sabouraudii, T. sulfureum)* also grow slowly. Colonies develop deep central crater-like depressions and varying degrees of yellow pigment. Typical Trichophyton microconidia and macroconidia are produced in most species, but these species too should be referred to a competent mycologist for final identification.

3. *Epidermophyton.*—A single species, *E. floccosum,* has a markedly characteristic microscopic pattern. Thick, blunt macroconidia with 2-3 septa are produced in clusters (Fig. 3). There are no microconidia. The gross colony which develops short aerial hyphae has a distinctly greenish cast. Only skin and nails are attacked by this species.

It should be emphasized that characteristic spore forms are most readily seen in primary isolates. Many species tend to lose their identifying spore structures after several transfers, making it virtually impossible to name the species from the resultant sterile hyphae. Since detailed descriptions of colonial characteristics can be found in the classification manuals,[1,2] they have been intentionally omitted in this outline. Gross differences which are of considerable value to the trained eye, vary with age of the culture and the type of medium on which it was grown. Usually such information is used only as an adjunct to, and rarely as the primary criterion for identification.

There are also several fungus diseases caused by organisms generally considered to be saprophytic but which occasionally produce lesions on the skin or hair. Since these fungi attack only the most superficial layers of skin and do not invade but only surround the hair shaft, the diseases produced by them are commonly referred to as superficial mycoses. This minor distinction merely serves to separate them clinically from the dermatomycoses which are caused by the well-defined group of fungi outlined above. Diagnosis can be established by characteristic microscopic structures seen in clinical material. Culture is not necessary and is rarely successful even if attempted. *Malassezia furfur,* the causative agent of tinea versicolor, *Nocardia tenuis* of trichomycosis and *Piedraia Hortai* and *Trichosporon Beigelii* of black and white piedra respectively are examples of such organisms. Complete descriptions of these fungi are given in the classification manuals.[1,2] They should be considered in all infections of hair and skin.




**II. Subcutaneous and Systemic Mycoses**

The second large category consists of organisms which cause systemic or potentially systemic mycoses. They are found in sputum, pus, gastric washings, blood, bone marrow, spinal fluid, vaginal discharges, biopsy specimens, urine and feces. In short, where systemic mycosis is suspected, no body exudate or tissue is beyond suspicion. Furthermore, most of these organisms have characteristic morphological structures in clinical materials which differ radically from the characteristic structures seen in

culture. It is essential to know both tissue (clinical material) phases and culture forms.

For further amplification, the organisms are divided into four sub-groups.

1. *Bacteria-like (Actinomyces) organisms.* There are at least six Actinomyces species considered to be pathogenic for man. Only three of the more commonly encountered ones will be discussed. Of these two are aerobic (Nocardia) and one is a facultative anaerobe (Actinomyces). Any one of the three species may appear in pus as "sulfur-yellow" granules composed of gram-positive tangled filaments. Each filament approximates the width of the tubercle bacillus (hence the name bacteria-like). The organized granules are generally replaced by simple gram-positive branching filaments in sputum or spinal fluid. When either filaments or granules are present, one of the following three species may be of etiologic significance:

a. *Actinomyces bovis.* Partial anaerobiosis and incubation at 37° C. are essential to the isolation of the organism. While it may be isolated on a number of enriched media, the characteristic identifying growth pattern occurs in veal infusion glucose agar (pH 7.6-7.8) shake tubes. A ring of small, white, fuzzy or lobulated colonies 3-4 mm below the surface of the agar appears after 3-4 days of incubation. Larger colonies may be dispersed through the deeper portion of the medium. All colonies are composed of delicate gram-positive branching filaments which may easily fragment into bacillary elements upon manipulation.

b. *Nocardia asteroides.* This organism, a strict aerobe, is isolated and cultured on glucose agar (pH 7.6-7.8) at room temperature. Colonies are glabrous (without aerial mycelium), irregularly folded and vary in color from pale yellow to deep orange. Microscopically these are composed of gram-positive branching filaments (Fig. 4). With careful staining they are also acid-fast. The acid-fastness of the organism can best be demonstrated in human clinical materials or in pus from lesions of experimentally infected animals. *N. asteroides* does not withstand concentration methods employed in the isolation of the tubercle bacillus.

c. *Nocardia madurae.* This species is cultured in the same way as *N. asteroides.* Colonies are glabrous and wrinkled, but the pigment produced varies from pink to red. The organism is not acid-fast. It is usually isolated from the disease known as "Madura Foot."

2. *Yeast and Yeast-like organisms.* There are two species in this sub-group which are recognized as definite pathogens. Neither has fastidious growth requirements and white to cream colonies of bacterial consistency are usually well-developed within 48-72 hours on routine laboratory media. Colonies can be easily picked with a bacteriological loop and make evenly turbid saline sus-

pensions. No aerial (cottony) hyphae are produced. The organisms reproduce by budding.

a. *Cryptococcus neoformans.* This unicellular species appears in clinical material as a single budding, round to oval (5-20u in diameter) yeast which is surrounded by a wide gelatinous capsule. Demonstration of this capsule in spinal fluid is diagnostic. An identical microscopic picture is presented in cultures of the organism. The capsules, however, can be seen in cultures and in many clinical materials only when the organisms have been mixed with India ink (Fig. 5). They may not appear in culture until after a week or two of incubation or until after a series of transfers has been made. Incubation at 37° C. accelerates their production. Many strains, but not all, are distinctly mucoid and gelatinous. The organism is most frequently isolated from spinal fluid.

Non-pathogenic strains of *C. neoformans* fail to grow at 37° C and are not pathogenic for mice.

b. *Candida (Monilia) albicans.* This is the only species to which both systemic and superficial infections may be attributed. It is also the only species which consistently presents itself as a secondary invader. The isolation of *Candida albicans,* particularly from sputum or pus, is not evidence that this organism is the actual agent of disease, but since it is capable of pathogenicity it must be identified and reported whenever it appears.

It occurs in clinical materials as a round or oval budding yeast (2-4u). Broad mycelial elements, described as pseudomycelia, are also present. *C. albicans* exhibits a special carbohydrate fermentative pattern, producing acid and gas in glucose and maltose, acid in sucrose and no reaction in lactose. When cultured on corn meal agar round chlamydospores are developed at the tips of branched, tree-like pseudomycelia (Fig. 6). Although it is a matter of some controversy, many workers consider the production of chlamydospores as being diagnostic.

3. *Yeast-mold organisms.* Four species appearing in clinical specimens as budding yeasts of varying size and shape are placed in this sub-group. By special cultural procedures and incubation at 37° C. they can also be isolated and maintained in the yeast phase. Colonies in this phase are soft, pasty and of a bacterial consistency. The same organisms grown at room temperature develop as white cottony molds in three of the species *(B. dermatitidis, B. brasiliensis, H. capsulatum)* and as waxy, tan to black colonies in the fourth *(S. schenckii).* Microscopically there is a profusion of branched hypae from which the specialized spores characteristic of each of the species are produced. It is frequently advantageous to culture the organisms in both phases, but as the identifying spores with one exception—*B. brasiliensis,* are produced only during

room temperature incubation, culture at this temperature is essential for exact identification.

a. *Blastomyces dermatitidis.* In clinical specimens and in yeast phase cultures the organisms appear as single budding, spherical cells (8-15u) with thick refractile walls.[6] In the mycelial phase, single small, round to oval spores are attached directly or by very short, fragile stems to the main hyphal elements (Fig. 7). Care must be exercised to ascertain that this is the only spore produced, since *Histoplasma capsulatum* presents a similar microscopic picture in an early stage of growth.

b. *Blastomyces brasiliensis.* This organism appears as a single and *MULTIPLE-BUDDING* (diagnostic form) yeast in clinical materials and yeast-phase cultures. The mycelial phase is singularly lacking in characteristic spore forms. Identification is made from the multiple-budding forms seen in yeast-phase cultures (Fig. 8).

c. *Histoplasma capsulatum.* Highly characteristic "tuberculate" chlamydospores (Fig. 9) arising only in the mycelial phase instantly differentiate this species from all others. They are produced *in addition to* the small spores described under *B. dermatitidis* and in primary isolates are the predominating spore form. Many strains can also be maintained in the yeast phase where again the very small (2-4u) oval cells similar to those seen in clinical materials reproduce by single budding.[7] Since this phase is fastidious in its growth requirements a higher percentage of isolates is obtained at room temperature incubation.

d. *Sporotrichum schenckii.* Colonies grown at room temperature are not filamentous, but waxy and membranous. Color varies from light tan to black and may change from one extreme to the other upon subsequent transfer of the same strain. Microscopic preparations from such colonies reveal delicate branching hyphae that bear oval, round or pyriform spores (conidia) laterally or in flower-like groupings from the ends of lateral branches (Fig 10). In the yeast phase,[8] the cells are elongated with sharply pointed tips from which the budding usually occurs. Similarly shaped cells, though rarely demonstrated, occur in human lesions.

4. *Molds.* Tissue forms of this group are quite distinct from the characteristic, identifying structures seen in cultures. Attempts to culture this group of organisms in the tissue phase have generally been unsuccessful. They are always incubated at room temperature and grow as typical cottony molds.

a. *Coccidioides immitis.* (CAUTION: Coccidioidomycosis is contracted by inhalation of spores of *C. immitis.* The laboratory where cultures are carelessly handled is a source of danger to all who chance upon or near it.) Suspected materials should be carefully examined for thick-walled non-budding spherical bodies (20-80u in diameter) containing endospores (2-5u). Immature spherules (without endospores) cannot be used as a diagnostic criterion.

Colonies which are at first cottony and white may become light brown with age. Aerial mycelium is rarely uniformly distributed over the surface of the medium and because of this, colonies often have a "moth eaten" appearance. Microscopically, segments of the broad and extremely septate hyphae wall off into barrel-shaped spores (arthrospores) that are easily detached from each other and dispersed (Fig. 11). Racquet hyphae are also seen in this species.

b. *Hormodendrum pedrosoi, Hormodendrum compactum, Phialophora verrucosa.* These three closely related organisms are agents of the subcutaneous infection known as chromoblastomycosis and have an identical appearance in clinical materials. Clusters of dark brown, thick-walled cells which *reproduce by splitting,* rather than by budding, may be representative of any one of the three species. The dark brown to black colonies produced on glucose medium are also similar in appearance. Species differentiation is made solely from the microscopic pattern. The pathogenic species must also be differentiated from Hormodendrum species which regularly and ubiquitously appear as contaminants. Final classification, in most instances, should be made by a mycologist.

*Hormodendrum pedrosoi* has three types of sporulation:

(1) Small spores (conidia) in branching chain formation from conidiophores (specialized hyphae on which conidia are developed) of varying length—Hormodendrum type (Fig. 12a).

(2) Conidia surrounding swollen, knotted, club-shaped terminal ends of hyphae—Acrotheca type (Fig. 12b).

(3) Conidia produced from flask-shaped conidiophores with a terminal cup—Phialophora type (Fig. 12c).

*Hormodendrum compactum* differs microscopically from *H. pedrosoi* by having chains of subspherical spores in closely packed spore heads.

*Phialophora verrucosa* produces conidia from flask-shaped conidiophores with a terminal cup. This is the only type of sporulation seen in this species (Fig. 12c).

c. Other molds which should always be kept in mind are the various species associated with maduromycosis. Workers should be constantly alerted for the presence in pus of white, black yellow or red granules. Such granules are composed of broad (2-4u) segmented hyphae rather than the delicate filaments seen in actinomycotic granules. Maduromycotic granules are always cultured on glucose agar at room temperature. The organism isolated may be any one of a number of species, most of which are ordinarily presumed to be saprophytic i.e. *Aspergillus, Penicillium, Cephalosporium, Monosporium,* etc.

Except for the agents of Rhinosporidiosis and Geotrichosis which are entities of extreme rarity, the above named species,

superficial and systemic, are the known pathogenic fungi. One can be relatively certain that if he becomes familiar with these species he will seldom fail to identify an organism of mycologic importance. Only under extreme circumstances, which must of course be evaluated by the clinician in charge, should any other fungus agent be indicted as being pathogenic for man.

## Methods

*Treatment of specimens:* Specimens of thick sputum and pus should be examined and inoculated as received. Solid stool specimens are diluted and emulsified in normal salt solution for examination and plating. Spinal fluid, pleural fluid, gastric washings and liquid sputum, pus and feces specimens are centrifuged until the supernatant is visibly clear. Direct microscopic mounts are prepared and media inoculated from the sediment. Biopsy specimens are ground in sterile mortars, diluted with minimal amounts of saline and plated directly. Blood and sternal marrow are also cultured as received.

Small flakes of skin and nails, or *hair roots* are placed at one-half inch intervals over the medium. Moistening the inoculating wire facilitates the handling of such specimens.

*Direct microscopic examination of clinical specimens:* Hair, skin, nails and thick pus are examined in 10% KOH. A small portion of the suspected material is placed in a drop of 10% KOH on a slide, covered with a cover slip and gently heated over the pilot light of a Bunsen burner. The cellular elements are dissolved while the fungus forms remain intact. The preparation is examined immediately.

Spinal fluid should be centrifuged at low speed for five minutes and the supernate poured off. A loopful of sediment and a loopful of India ink (diluted with water or salt solution if desired) are mixed together on a slide, covered before drying with a cover slip and examined immediately.

Organisms are best demonstrated in many clinical materials by wet preparations. These are made by placing a loopful of the material or centrifuged sediment thereof on a slide and covering it with a cover slip for immediate examination. This type of preparation is used more than any other in the direct microscopic examination of clinical materials for systemic pathogens.

The following stains are of value under the conditions specified:

1. Gram stain in clinical materials and cultures of suspected actinomycosis and moniliasis.

2. Acid-fast stain in clinical materials and culture in suspected nocardiosis.

3. Giemsa or Wilson stain for peripheral blood smears and bone marrow in suspected histoplasmosis.

4. India ink preparations of clinical materials and cultures in suspected cryptococcosis.

*Microscopic examination of cultures:* The importance of properly preparing microscopic mounts of cultures for direct examination cannot be over-emphasized. For demonstration and teaching purposes slide cultures[2, 9, 10] that show the undisrupted attachment and arrangement of specialized spore to hypha are, of course, ideal. Much can be learned, however, from good preparations made directly from the gross colony.

A small fragment of aerial mycelium is transferred to a drop of lactophenol,* saline or water on a slide by means of a straight, strong wire attached to a bacteriological loop handle (dissecting needles work equally well). With a second straight wire the material is gently removed from the first needle and teased apart in the mounting fluid. A cover slip is then put in place. If lactophenol has been used as the mounting agent the preparation should be heated over the Bunsen burner pilot light. This will drive the stain into the fungus filaments and at the same time remove air bubbles which may have been present. A thin triangular section of agar removed from the edge of the colony should be examined in the same way. In carefully prepared mounts, many of the spores remain attached to the hyphal elements and identification can be made immediately. Preparations made in this fashion will also serve as permanent mounts if the edges of the cover slip are sealed with ordinary finger nail polish.

*Culture medium:* For many years the most difficult part of medical mycology has been the isolation, not the identification, of pathogenic fungi.

Workers lacked concentration methods similar to those employed for the isolation of the tubercle bacillus so that the subsequent overgrowing of media by rapidly growing bacterial and saprophytic fungus species in heavily contaminated materials invariably reduced the chance of isolating any of the slower growing fungus pathogens. Adjusting media to acid or alkaline pH levels and incubating at room temperature only partially overcame the difficulty. Many bacterial and saprophytic fungus species continued to flourish and mask the presence of possible pathogenic mycotic organisms.

However, the antibiotics, penicillin and streptomycin,[11] have proved to be excellent weapons against this technical obstacle.

---

* Lactophenol

| | |
|---|---|
| Phenol crystals | 20 gms. |
| Lactic acid syrup | 20 ml. |
| Glycerol | 40 ml. |
| Water | 20 ml. |
| Cotton blue | 0.10 gm. |

Cotton blue is added after the other ingredients have been melted and mixed.

The addition of 25 units of penicillin and 40 units of streptomycin, each per ml of medium, adequately inhibits the growth of gram-positive and gram-negative bacterial organisms, but has no inhibitory effect upon the growth of any of the pathogenic fungus species except the Actinomycetes. These antibiotics, therefore, cannot be incorporated in media employed in the isolation of agents of suspected actinomycosis. For all others, it is a recommended procedure since under certain cultural conditions streptomycin, at least, not only does not inhibit but actually enhances growth of several pathogenic species.[12]

The advantages of adding the antibiotics are two-fold:

1. It is no longer necessary to attempt isolation on medium of extremely high or low pH, a procedure which retards growth of *H. capsulatum* and *B. dermatitidis* as effectively as it does some bacterial species.

2. Media can be heavily inoculated, thereby increasing the possibility of isolation. Instead of inoculating a single loopful of material, plates can now be flooded with one or more ml. of the submitted specimen or heavily streaked with a cotton swab.

Thus, in addition to Sabouraud's and potato glucose agar* which are still the media of choice for production of characteristic spore forms after isolation many other media (meat infusion agars, glucose agar, blood agar, etc.) are also satisfactory for the isolation of pathogenic fungi if the antibiotics are first incorporated. Brain heart infusion agar (Difco)[13] containing 1% glucose and the antibiotics has been found to be especially satisfactory for the isolation of *H. capsulatum* and *B. dermatitidis*.

Clinical specimens suspected of containing systemic fungi are inoculated on meat infusion agar (pH 6.8-7.4) enriched with blood and glucose and incubated at room temperature. A duplicate set is incubated at 37° C for the yeast phase isolation of organisms in the yeast-mold group. Both sets should contain the antibiotics in the amounts specified. Plates should be sealed with "Parafilm" or some similar substance to retain moisture and reduce the possibility of external contamination. If, after two weeks of incubation, no colonies have appeared at 37° C, this set of media should be removed to room temperature and observed for an additional two weeks. Colonies at either temperature should be transferred as soon as they appear to potato glucose agar slants and incubated at room temperature for the production of characteristic identifying spores. Saprophytic fungus species can thus be eliminated quickly from considera-

---

* Cut 300 gms whole potato into small pieces (1 inch blocks), steam in 500 ml. distilled water for 30 minutes. Strain through a moist cloth, make up to volume, add 1% glucose and 1.5% agar. Heat to dissolve, tube for slants, and autoclave at 15 lbs. for 20 minutes. No pH adjustment necessary.

tion and the isolated pathogen removed to an atmosphere devoid of competition from contaminating fungus species.

Suspected actinomycotic materials should also be incubated in duplicate sets on glucose or blood agar (pH 7.4-7.8) which does not contain the antibiotics. In this instance one set is incubated anaerobically at 37° C for the isolation of *Actinomyces bovis* and the second aerobically at room temperature for the two pathogenic Nocardia species. Thioglycollate medium is satisfactory for the culture but not the isolation of *A. bovis*.

Even though isolates may appear as early as 48 hours after inoculation, all media should be incubated from 4-6 weeks before being discarded as negative for pathogenic fungi. In most instances, colonies become evident within the first or second week, but for those species which are extremely slow growing the longer incubation period must be fulfilled. Isolation of pathogenic agents is always enhanced by the use of freshly prepared, moist medium, which should be of sufficient depth to compensate for the dehydration that will occur during the long period of incubation.

Littman[14] has devised a special isolation medium which utilizes crystal violet and streptomycin for the inhibition of bacterial species and oxgall for the limitation of saprophytic fungus colonies. It is satisfactory for isolation of all of the pathogenic species except the Actinomyces, *H. capsulatum* and *B. dermatitidis*. Isolated colonies should be transferred to potato glucose agar for study of specialized spore characteristics.

Formulae for specialized media employed in the differentiation of Candida species (carbohydrate base, corn meal agar, carrot agar, etc.) are given in the classification manuals.[1,2]

*Animal inoculation:* This is of little practical value in the study of the dermatophytes. It is used chiefly for confirmation of cultures of systemic pathogens, but in many instances direct inoculation with clinical material is also helpful. Except in *C. neoformans* (pathogenic strains), *C. albicans* and *N. asteroides* fungus species rarely cause death in experimentally infected animals. Heavy saline suspensions of yeast-phase cultures are used wherever possible for injection as they are not only more easily manipulated but also tend to be more pathogenic. Table II lists the animal of choice, route of injection, period of incubation, etc., for those organisms in which animal inoculation materially contributes to the study of a species.

## Discussion

It should be repeated that the foregoing classification has been presented as a guide for those workers who have not yet been initiated into the field of medical mycology but who must from time to time include the study of pathogenic fungi in their rou-

**TABLE II**

**Animal Inoculation with Pathogenic Fungi**

| Organism | Animal of Choice | Inoculation Route | Exposure Interval | Gross Pathology (lesions) | Microscopic Findings |
|---|---|---|---|---|---|
| N. Asteroides* | Guinea Pig | Intraperitoneal | 8–10 Days | Mesentery Peritoneum | Acid-fast branching filaments |
| C. Neoformans* | Mouse | Intraperitoneal | 1–4 Weeks | Peritoneum, brain, spleen, gelatinous masses in mesentery | Budding encapsulated yeasts |
| C. Albicans | Rabbit | Intravenous | 4–6 Days | Abscesses through out peritoneum and kidney | Thin-walled budding yeasts with or without pseudomycelia |
| B. Dermatitidis* | Mouse | Intraperitoneal | 2–3 Weeks | Liver, spleen, lungs, lymph nodes, peritoneum | Single-budding cells with thick refractile walls |
| B. Brasiliensis* | Mouse | Intraperitoneal | 4–6 Weeks | Spleen, liver, diaphragm, mesentery | As above, except many cells have multiple buds |
| H. Capsulatum* | Mouse | Intraperitoneal | 2–3 Weeks | Mesentery, diaphragm, visceral organs | Small, oval budding cells |
| S. Schenckii* | Male rats | Intratesticular | 1–4 Weeks | Peritonitis, orchitis | Cigar-shaped budding cells |
| C. Immitis | Male Guinea Pig | Intratesticular | 10–15 Days | Orchitis | Double-walled spherical bodies containing endospores |

* It is advisable to inoculate 6–12 mice and 1–3 guinea pigs. Mice should be sacrificed and posted at 2–3 day intervals beginning two weeks after inoculation. Guinea pigs should be sacrificed at the end of one week and at weekly intervals thereafter if death has not meanwhile ensued.

tine curriculum. By so organizing and sub-grouping the fungi that are pathogenic for man it is hoped that the subject will seem less complex and thus serve as an incentive to further study of these organisms.

In all instances the facts given in this outline should be substantiated and enlarged through study of more complete and detailed works.

## Summary

1. A practical classification of fungi pathogenic for man has been outlined.

2. Technical methods employed in the direct microscopic examination of clinical materials and cultures have been described.

3. Methods of culture, culture media, and animal inoculation have been briefly discussed.

## BIBLIOGRAPHY

1. Conant, N. F., Martin, D. S., Smith, D. T., Baker, A. D., and Callaway,

J. L.: Manual of Clinical Mycology. W. B. Saunders Co., Philadelphia, 1944.
2. Lewis, G. M., and Hopper, M. E.: An Introduction to Medical Mycology. The Year Book Publishers, Inc., 2nd ed., Chicago, 1943.
3. Henrici, A. T. (Skinner, C. E., Emmons, C. W., and Tsuchiya, H. M.): Molds, Yeasts and Actinomyces. John Wiley and Sons, Inc., 2nd ed., 1947.
4. Swartz, J. H.: Elements of Medical Mycology. Greene and Stratton, New York, 1943; 2nd Edition, New York, 1949.
5. Hazen, E. L.: Microsporum Audouini: The effect of yeast extract, thiamine, pyridoxine and *Bacillus Weidmaniensis* on the colony characteristics and macroconidial formation. Mycologia, **39**: 200, 1947.
6. Martin, D. S., and Smith, D. T.: The Laboratory Diagnosis of Blastomycosis. J. Lab. and Clin. Med., **21**: 1289, 1936.
7. Conant, N. F.: A cultural study of the life cycle of *Histoplasma capsulatum*, Darling, 1906. J. Bact., **41**: 563, 1941.
8. Campbell, C. C.: Use of Francis' glucose cystine blood agar in the isolation and cultivation of *Sporotrichum schenckii*. J. Bact., **50**: 233, 1945.
9. Johnson, Edwin A.: An improved slide culture technique for the study and identification of pathogenic fungi. J. Bact., **51**: 689, 1946.
10. Littman, M. L.: A special microslide for culture of fungi. Am. J. of Clin. Path., **19**: 278, 1949.
11. Thompson, L.: Notes on a selective medium for fungi. Proceedings of Staff Meetings of Mayo Clinic, **20**: 248, 1945.
12. Campbell, C. C., and Saslaw, S.: Enhancement of growth of certain fungi by streptomycin. Proc. Soc. Exp. Biol. and Med., **70**: 562, 1949.
13. Howell, A., Jr.: The efficiency of methods for the isolation of *Histoplasma capsulatum*. Public Health Reports, **63**: 173, 1948.
14. Littman, M. L.: A culture medium for the primary isolation of fungi. Science, August 1, 1947.

## A RAPID SCREENING TEST FOR RH SENSITIVITY*

ALVINA M. THOMPSON, M.T. (ASCP), *Casper, Wyoming*

When a large number of serums are being tested for Rh sensitivity, there can be a great consumption of valuable time. Much of this time can be saved if a rapid screening test is employed to determine qualitatively the existence of any degree of sensitivity. This type of test is used in Dr. H. Wallerstein's laboratory to help eliminate the titration of Rh negative serum in which no sensitivity to the Rh factor exists. The test that is to be presented is a modification of the test used in that laboratory.

The test itself is very simple and requires only a few biologicals which are easily obtained. They are: the serum to be tested, oxalated blood cells containing all three Rh factors, 30% Bovine albumin (Armour) and the viewing box used in the rapid slide test. It is a rapid slide test following the same pattern as that described by Diamond and Abelson for the demonstration of agglutinins in Rh sensitivity. However, the addition of bovine albumin makes the test more sensitive and demonstrates both blocking antibodies and conglutinins in addition to agglutinins.

* Read before ASMT Convention, Roanoke, Va., June, 1949.

The actual technic is as follows:

1 drop —serum to be tested for sensitivity
1 drop —30% Bovine albumin
2 drops—oxalated CDE blood cells

These are placed on a slide, then mixed with an applicator or glass rod and spread over an area approximately 1 inch in diameter. It is then placed in the warm viewing box and slowly rocked back and forth for exactly two minutes. It is essential that the slide is read at the end of two minutes because drying around the edges may simulate a false sensitivity. A weak reaction will only show a fine clumping on the edge of the blood. Any degree of sensitivity will show as a granulation or clumping of the cells. If any sensitivity is observed, the serum can then be titrated for agglutinins, blocking antibodies, and conglutinins.

While the technic appears simple, there are several sources of error that will affect the accuracy and the sensitivity of the test. The drops used for all of the biologicals should be approximately the same. The viewing box should be at least 40° C. The slide should be rocked very slowly. The blood cells should not be over-oxalated. The anticoagulant of the Wintrobe sedimentation test used in the same proportions has proved very satisfactory. The cells should be well concentrated. If necessary, they may be concentrated by decanting the plasma until a sufficiently heavy suspension is obtained. The cells may be used as long as no visible hemolysis is present. These cells should be the pooling of at least ten known Rh positive bloods, which may be obtained from the blood sedimentation tests, or if these are not available, the cells of a known CDE individual may be used.

The test has the advantage of obtaining positive results in all cases of Rh sensitivity whether only agglutinins exist or only blocking antibodies and conglutinins are present. While the degree of sensitivity and the degree of erythroblastosis do not always agree in any test, this test is sensitive enough so that it is positive in all cases of erythroblastosis even those showing only a slight degree of jaundice. The babies, of a large number of these cases giving a weak positive reaction, require no treatment whatsoever. The reaction acts as a warning of a possible marked sensitivity in the next pregnancy. This is very important so that the subsequent pregnancy can be more closely observed and the baby treated according to the degree of sensitivity.

In one of the recent cases observed, this test gave an increasingly stronger positive reading. This serum, on titration, showed no agglutinins but the final titration results were blocking antibodies 1-32 and conglutinins 1-512. The baby when born was anemic, showed a mild jaundice which increased upon birth and has a high erythroblastic count. The baby was immediately

given a replacement transfusion and survived with no serious complications.

In contrast, let us observe the results on a Rh negative patient, in her fourth pregnancy, with no apparent sensitivity to date. The first three children were all Rh positive and had had no jaundice or other evidence of erythroblastosis. There was no sensitivity observed during the first five months of the present pregnancy. During the sixth month a weak sensitivity appeared which did not increase during any of the subsequent tests. The baby when delivered at full term was Rh negative. The reaction observed, according to Potter, may be a true anamnestic reaction caused by some condition existing in all pregnancies and not necessarily related to the antigenic action of Rh positive fetal cells. The weak sensitivity observed was apparently residual to a previous pregnancy. This serum when titrated with 30% Bovine albumin gave a weak 1:1 conglutination reaction.

This rapid slide test, while simple and accurate, through the addition of 30% Bovine albumin, will detect blocking antibodies and conglutinins besides agglutinins.

## REFERENCES

1. Potter, Edith: Rh (Chicago—The Year Book, Inc., 1947) —286.
2. Davidsohn: The Rh Factor (New York—Grune & Stratton, 1948) —139.
3. Wallerstein: Personal Communication—April, 1948.
4. Diamond & Abelson: The Demonstration of anti-Rh agglutinins: An accurate and rapid slide test—J. Lab. & Clin. Med. 30:204, March, 1945.
5. Wiener, A. S.: Conglutination Test for Rh Sensitization—J. Lab. & Clin. Med. 30:662, August, 1945.

# A COMPLETE METHOD FOR CONSISTENT BLOOD STAINING

WALTER T. CASE, M.T. (ASCP), *Batesville, Arkansas*

The use of the stained blood film for diagnostic and prognostic purposes has attained universal acceptance. Enumeration and perusal of the stains so employed leads one to suspect, from their number, similarity, and modification, that results in the hands of different technicians vary considerably.

The intent of this article is not to discuss the merits of the various stains nor to present a mass of data, but to make available an excellent blood stain, which is the result of a series of private experiments and refinements to the end that its manufacture and use are sure and simple.

The stain, an unrefined ammonium polychrome of methylene blue combined with eosin, has been prepared and used for a period exceeding seven years. At least ten thousand slides have been stained and examined. Freedom from precipitates and remarkably good staining of cellular elements have been noteworthy. Malarial protozoa, polychromasia, and stippling are

readily observed without special treatment. Bottle failure has not been encountered.

A full description of the method is offered.

**Materials**

| | |
|---|---|
| Methylene blue, chloride, certified or USP..... | 1.0 gm |
| Eosin, yellow, certified........................ | 1.0 gm |
| Ammonia water, stronger, USP................ | 2.0 ml |

**Apparatus**

| | |
|---|---|
| Balance and weights | Dropper pipette |
| Watch glass, 100 mm | Applicator stick |
| Watch glass, 75 mm | Spatula, steel |
| Autoclave, steam | Mortar and pestle |

**Process**

The weighed dyes are heaped in the center of the larger watch glass. Stronger ammonium hydroxide, approximately two mls, is added drop by drop while the dyes are mixed with the applicator stick to a uniform wetness throughout. Exacting measurement of the ammonia is not necessary as two mls is in excess of the actual requirement. Cover the mass with the smaller watch glass and submit the mixture to fifteen pounds steam pressure, 121° C., in the autoclave for fifteen minutes. The steam is then exhausted rapidly, the covering watch glass is removed, and the preparation is allowed to dry overnight in an incubator. Scraping the material into a mortar and grinding to a fine powder completes the process. The resulting stain combination is relatively stable if kept in a vial and stoppered sufficiently tight to prevent exposure to acid fumes. Larger quantities of the stain are prepared similarly.

A comparison of this process to those of Wright, Jenner, Balch, and others will disclose that the use of ammonium hydroxide eliminates the careful calculation of the correct amount of alkali needed for polychroming and avoids the lengthy filtration for dye recovery. These considerations prompted its inception. Previous reference to this polychrome has not been found and its use is thought to be original.

**Staining Solution**

| | |
|---|---|
| Prepared stain, dry, powdered............... | 0.3 gms |
| Acetone,[2] C.P. ............................. | 5.0 mls |
| Methanol, absolute ......................... | 95.0 mls |

The staining solution is readily prepared by placing the components in a screw-capped bottle which allows agitation in a Kahn shaker or vigorous shaking by hand. The aging period

of this solution is about seventy-two hours, but it may be used immediately if the exposure of the blood film to the stain is increased to one and one half times the regular staining period. Filtering to remove the excess dye is unnecessary.

## Staining Method

A thin blood film is selected and placed on a level staining rack. Exactly ten drops of the staining solution are slowly distributed in a manner as to cover the entire surface of the slide. Thirty seconds after counting the last drop, the slide is removed from the rack and dropped into a container of distilled water. Ninety seconds later the slide is removed from the water and rinsed in a second container of acetone, C.P. Three or four unhurried in and out excursions in an interval of fifteen seconds suffices to remove a overstain of the erythrocytes. The acetone is in turn rinsed from the slide in a third container of distilled water. Upon drying, the blood film is ready for examination.

Mention should be made that the use of more than ten drops of stain will require a longer staining time. Also, that there is no danger of over-decolorizing by a single passage of a film through the water, acetone, water steps. The utilization of coplin jars as containers allows the repeated use of the acetone, evaporation and loss being replaced as required. The action of distilled water is satisfactory without the addition of buffer substances, and need not be changed with each slide though it is desirable to do so daily. Chlorinated tap water is very unsatisfactory with this stain.

Results with the technic just described have been so gratifying with single slides that it is unhesitatingly recommended to replace the one initially employed, the fixation-dilution method in use with Wright type stains.

For multiple staining, Chanco's immersion technic,[1] after which the preceding was patterned, is accomplished by the substitution of a stain-filled coplin jar to replace the staining rack. The blood films are then immersed in the stain for forty to sixty seconds as the initial step of the foregoing procedure. The specified timings are nearly minimal. The staining characteristics displayed are subject to the time allowed for the first water exposure. Consistency of results is obtained by adherence to a specific timing.

## Summary

1. A simple process for the production of ammoniated methylene blue-eosin blood stain has been presented.

2. A suitable staining solution has been recommended with alternate procedures for single or multiple staining of blood films.

## REFERENCES

1. Elton, N. W., Fredenburgh, E. J., and Manning, D. W.: The Chanco technic in Wright's stain, Tech. Bulletin, Reg. Med. Technologists, 18:92-93, 1948.
2. Lillie, R. D.: Some experiments on the Romanovsky staining of blood films, J. Lab. and Clin. Med., 28:1872-1875, 1943.


# MICROSCOPIC TECHNIQUE WITH SPECIAL REFERENCE TO PHASE MICROSCOPY

FRANCIS W. PORRO, M.D.*

The microscope is perhaps the most essential of the precision instruments used in the laboratory. We are fortunate in having excellent microscopes available. While in general they are quite successfully used, there are many ways in which more efficient use may be made of them, and they also deserve better care than they commonly receive. This discussion will refer only to the common types of medical microscopes.

Inclined binocular eyepiece holders are well known and result in the most comfortable arrangement for use of microscope over extended periods of observation. These are furnished with paired eyepieces so that the two fields are equal when both of the eyepieces are adjusted to the same focal plane, one of the tubes being made movable for this adjustment. Unfortunately, it is quite common to find that the movable tube is left in such a position that the image on that side is blurred or unequal in field to the other side when the fixed side is in focus. The result is that although the observer is often unaware of it, he is disregarding the image on the unadjusted side and is being subjected to unnecessary eye strain. The remedy for this is to focus with the side having the fixed tube length and adjust the other side to correspond.

It is not so well known that inclined monocular eyepiece holders are also available and that the use of one of these with a monocular microscope results in much greater working comfort. They work much better if a black eye shield is used, such as are available from the microscope manufacturers.

Most observers prefer the 10X eyepiece, but for many purposes it is inferior to the 5X or 6X eyepiece furnished with the microscope and which should be kept at hand for immediate use when needed. These are particularly useful in searching fields in which the number of cells or organisms sought for is small; for instance, in doing differential counts in leukopenia, searching for acid fast and other bacteria, etc. The real field covered by these smaller magnifying eyepieces is at least twice that of the ones of larger magnitude.

* Pathologist to St. Mary's Hospital, Evansville, Indiana.

There are some special eyepieces that should have wider use, particularly those with high eye points so that glasses can be worn comfortably while looking through the microscope. Persons with astigmatism should use such eyepieces for prolonged observation. These eyepieces are made in the compensating variety only, but they work well enough with achromatic objectors and can be obtained in 8X, 10X, and 12½X sizes.

The proper use of lamps and condensers deserves more attention. The lamp selected should have a fairly broad, uniform light source, usually obtained by the ground glass in front of a clear glass filament lamp. Ordinary frosted bulbs are satisfactory if placed in a low position and properly shaded. An ideal lamp is one in which there is a condenser in the lamp housing, and it is better if there is also a field diaphragm. The beam of the lamp should be projected carefully to the center of the substage mirror. The small substage lamps are not bright enough for proper illumination of binocular microscopes when oil immersion is used.

In use it is always preferable to use the plain side of the mirror in microscopes having a substage condenser. In using the condenser, it is preferable to have the top of the condenser always near the slide and the best resolution of the image with oil immersion objectives is not possible unless this is done. It is preferable optically to adjust the light brilliance by use of the iris diaphragm rather than by lowering the condenser. It is not realized by many that the full resolving power of the oil immersion objective is not obtained unless there is oil between the condenser and slide as well as between slide and objective. This oil underneath the slide is not necessary for ordinary purposes, but for the most critical observation, for photography or for projection, it is of great assistance.

The ideal immersion medium shoulld have a refractive index equal to that of glass, but for most purposes this does not have to be exact. Heavy mineral oil is more convenient for ordinary use than the sticky cedar oil. If it is desired to accurately adjust the refractive index, it can be done by adding alpha-bromonaphthalene to the oil, stirring with a glass rod until the rod is difficult to see. Similar oil of proper refractive index may be obtained as Crown oil and as Shillaber's oil, the latter being made in two viscosities. It is preferable to use a medium of light viscosity with moist preparations; this is also preferable for use under the slide. Besides light viscosity oils, a lighter medium of proper refractive index is methyl benzoate which has the advantage of evaporating completely within a short time. It does have some odor.

Dark field microscopy deserves greater use than it now receives. By use of a dark field substage stop with low power ob-

servation of unstained material such as urinary sediment, fungi, large parasites, ova, etc., are often observed to greater advantage. For higher powers a dark field condenser is necessary and may be readily used with a little experience in adjustment. Most of these condensers are made to work with slides a little thicker than average, but ordinary slides can be used by turning down the condenser until the light in the preparation is a narrow beam rather than ring shaped. For illumination built-in lights are satisfactory; if the bulb should burn out and another is not available, the lamp housing is easily removed from the substage and a separate lamp can be used with the substage mirror and dark field condenser. Such a light should be as bright as possible, and where projection bulbs are used, the ground glass should be removed from the system. Oil should be placed between the condenser and slide and the beam adjusted as mentioned. The preparation is then focused under low power and centered. The high, dry power may now be used without funnel stop and is satisfactory for observation of most bacteria, including treepenoma. After observation under high power, oil immersion may be used by placing oil on top of the cover glass and using an oil immersion objective in which a funnel stop has been introduced into the objective. Oil immersion objectives can be purchased with an iris diaphragm instead, and this is preferable where dark field is to be used a great deal.

While built-on mechanical stages are most widely used, the writer prefers an attachable mechanical stage as these are equally satisfactory and are easily removed so that the plain stage can be used. Superior to either of these is the integral mechanical stage which can be obtained with some extra cost.

Recently equipment has been introduced for the practical use of light wave retardation for the formation of images of unstained objects observed through a compound microscope. This type of observation is called phase microscopy.

In medical laboratory practice most observations are made by bright field microscopy where light passes through the specimen and the image contrast and detail are obtained by differences in light transmission. The sharpness and detail are naturally improved by staining but are generally rather poor in most unstained material.

If the object is lighted by very oblique illumination so that the direct beams do not enter the objective, a bright image of reflected light from the object is seen against a dark background, hence dark field microscopy. This is very useful for fine, simple, unstained structures and is generally superior to bright field observation for such specimens.

In phase microscopy (also known as phase contrast or phase difference microscopy) an image of a more or less transparent

unstained object in a transparent medium is obtained by placing a substage stop in the lower focal plane of the condenser, shutting out the central rays and the peripheral rays and allowing a ring-shaped beam to pass through the condenser object and objective. In the back focal plane of the objective, a ring-shaped quarter wave retardation plate is placed (by the manufacturer) in the back focal plane of the objective to coincide exactly with the image of the condenser stop. The resulting image is dark against a light background and reveals much greater detail in unstained transparent objects than does light field observation. This is called a dark contrast image and is the type most commonly used. However, by reversing the position of the retardation ring in the objective, light images against dark backgrounds are obtained with greater structural detail than is commonly seen with dark field microscopy. This is called bright contrast and as of the present commercially available, I believe, from only one manufacturer.

Phase microscopy is useful in observing unstained, more or less transparent material, especially living cells, fungi, protozoa, parasites, etc. It can be used for routine work along these lines, but because of the expense of these accessories and the extra skill required in adjustments, its most promising use is in research observations on living micro-organisms and other cells. Attempts by the writer to use this method for counting chamber differential counts on blood and spinal fluid were not satisfactory because of the ghost images of the background material being brought out more prominently and obstructing the detail in the leukocytes. Fresh cell scrapings of tumors can be observed satisfactorily, but its use with sections was not successful. Photographic images of observed material are readily obtained.

The technique of using these accessories, which may be attached to a suitable microscope or bought with a microscope, is fairly exacting. A brilliant light source should be used. This is most conveniently obtained by one of the microscope lamps having a 100-watt projection bulb, lamp condenser, and iris diaphragm.

To begin with an object is focused upon without use of the substage ring stop and with the light dimmed by ground glass or neutral filter. The image of the light is then brought into the same plane as the specimen by focusing the lamp and microscope condensers. The substage ring stop is then placed, using one corresponding tothe objective to be used. The light made as brilliant as possible by removing ground glass or filter from the beam and with an observation telescope in place of the eyepiece, the centering screws on the condenser are moved until the ring of light coincides with the retardation ring in the back

focal plane of the objective. The observation telescope is then replaced by an ordinary eyepiece and the phase contrast image is seen. The fine adjustment can be used for focusing.

In conclusion a word should be added about the care of microscopes. When not in use the microscope should be covered by a dust cover, bell shield, or placed in its case. Light machine oil should be kept handy and the moving parts oiled occasionally. A little fine graphite placed on the sliding surfaces is helpful. Particular care should be taken to clean any cedar oil from objectives or stage by use of xylol or lens paper. For cleaning the outer lens surfaces, some prefer to use petroleum ether or benzine rather than xylol. For cleaning prisms or the back lens of objectives, brush lightly with a camel's hair brush.

## THE PRACTICE OF HEMATOLOGY IN GENERAL OFFICE MEDICINE

By CLARA M. BECTON, M.S., M.T.
*Petaluma, California*

The practice of medical technology in the office of a general practitioner is not limited to a blood count taken from the earlobe, a urinalysis, and an occasional blood sugar and possibly a non-protein-nitrogen determination.

In no field of medicine is differential diagnostic hematolo y of more import than in general practice. The busy doctor co ring all of the general fields, if his training and medical acumen are average and, if he is abreast of present day medical progress, is usually an expert diagnostician. Moreover, it is this class of physician who needs precision hematology. Office medical technology offers the opportunity for a perfect coordination between the physician, the patient and their relationship to the medical laboratory. By virtue of the physical examination, the clinician knows what tests are indicated; the laboratory examinations prove or disprove in vitro demonstrable manifestations of disease processes.

The practice of hematology as an art and a science carries with it a grave responsibility. Although limited to the study of various phenomena concerning the formation and destruction of the blood cellular elements and to the tests which depict numerical changes, morphological alterations, and other biological abnormalities of the blood cellular elements, the so-called erythrocytes, leukocytes, and thrombocytes, hematology is more than mere blood counting.

Hematological diagnosis depends largely on a series of tests from which the physician is enabled to derive an opinion from multiple rather than single observations. Much of the fascination in doing differential hematological studies is due to the fact that the tests which tie in with the dyscrasias follow fixed

patterns that are characteristic for the anemic, leukemic, and purpuric states. The following routine procedures for proving or disproving blood disorders have proven very practical in practice.

In anemia cases in addition to the routine blood count and serology the following routine has been adopted:

1. Hematocrit by the Wintrobe method with the calculations of the respective indices.
2. Reticulocyte count
3. Fragility
4. Determination of bilirubinemia
5. Sedimentation
6. Detailed study of the stained smear with a Cooke-Ponder differential, calculation of the absolute numbers of the various leukocytes, and a careful study for abnormality of the erythrocytes.

Any case showing purpuric manifestations usually receives these tests.

1. Estimation of platelets
2. Bleeding and coagulation times
3. Ivy capillary bleeding times in selected cases
4. Clot retraction
5. Prothrombin time
6. Coagulation time of recalcified plasma
7. Rumple-Leeds
8. Fibrinogen in selected cases.

Any blood count revealing blast cells without intermediary forms is strongly suggestive of a leukemia. The presence of abnormal white cells on a stained smear indicates a thorough leucosis study.

1. 300-1000 cell differential by the Cooke - Ponder method. (gives average segmentation per neutrophile)
2. Buffy Coat study. (Heparin is recommended as the anticoagulant)
3. Calculation of absolute numbers of cells
4. Sedimentation
5. Heterophile antibody in selected cases
6. Vital stains in selected cases
7. Platelet count in selected cases

A well-trained, experienced technician is requisite for expert hematology. She must be cognizant of the terminology used in the nomenclature of leukocytes set forth by the American Society of Clinical Pathologists. Definite fixed descriptions are very helpful in the classification of pathological cells. Perfectly stained smears must be obtained if accuracy is to be maintained. The following outline for cell identification has been very advantageous to the writer.

1. What is the size of the abnormal cell in its relation to the

normal erythrocyte (7 micra) and to the normal neutrophilic granulocyte (14 micra)?

2. What kind of a nucleus is present, what is its position and shape, and what is its size in its relationship to the cytoplasm?

3. What is the character of chromatin? Is it reticular, fine or coarse? Is it coarse, stranded or trabeculated? Is it pycnotic? Can para and oxychromatin be distinguished? Are nucleoli present?

4. What is the character of the cytoplasm? Is it clear, stippled, or opaque?

5. Are granules present or absent? What size are the granules? Are they neutrophilic, basophilic, or eosinophilic? Are they azurophilic?

If a nucleus shows nucleoli, fine reticular chromatin with para and oxychromatin frankly demarked, one is usually safe in assuming this is a myeloblast in contrast to the coarser chromatin of the lymphoblast and its fairly well defined nuclear membrane. Cytoplasm is usually scant in the myeloblast. The intense blueness of the cytoplasm of plasma cells and the clear perinuclear zone distinguish these cells.

The definitive granules of the pro-myelocytes and the nuclear remnants of nucleoli place these cells in their place. The ground-glass appearance of the cytoplasm and its tendency to show pseudopodia and the lacy fine reticular chromatin differentiate the promyelocyte from monocytes.

Trabeculated chromatin in a lymphocyte with the absence of nucleoli pidgeon-hole this type of cell as the pathological lymphocyte encountered in infectious mononucleosis. In the earlier stages of infectious mononucleosis a more monocytoid is frequently encountered.

By learning which cells have opaque cytoplasm (characteristic of pathological cells) nucleoli and those which have granules, and those which exhibit clear cytoplasm, one has a working approach toward accurate cell identifications.

If one is impressed by the density of both the nuclear chromatin and cytoplasm, the chances are that the cell in question belongs to the erythrocyte series.

In the practice of present day hematology the usefulness of the medical technologist is directly proportional to her training and ability to utilize time-saving technology. In as much as the value of routine hematology is dependent upon the dispatch of reports, it is essential that a simple uniform "routine" be used. As a measure of expediting routine work in general practice the following routines have been worked out. The patient is given a card that designates whether the patient is classed as surgical, medical, obstetric, pediatric, or minor surgery. The term diagnosis is used when liver function, kidney function, and blood

chemical studies are to be made and, of course the specific tests are ordered as indicated.

Surgical cases have a complete blood count including serology, bleeding and coagulation times, type and Rh if hemoglobin below 12 grams.

In addition to routine serology, medical and orthopedic cases receive a complete blood count with particular attention placed upon the Cooke-Ponder differential and a Sedimentation test by the Westergren method. Westergrens give a better graph in arthritic cases than the other commonly used methods.

Obstetrical cases have serology, complete blood count, type and complete rh typing if D antigen is lacking. A Wassermann and rh and type are done on all husbands.

Pediatric cases receive only a complete count done on capillary blood.

As a measure for maintaining uniform hematological reports, the following rules are observed.

1. Venous blood is used on all adults and children over 12 years.

2. Finger blood is used for children under 12 years and for partial repeat counts.

3. The plantar surface of the heel, or the toe is used as the puncture site in infants.

Repeat counts are done under the following circumstances:

1. If the count is in variance with previous counts.

2. If the counts do not agree within the accepted ranges error: namely, 3-5% hemoglobin and 300,000 red cells.

3. In any original count in which the color index is below 0.85 or above 1.05.

4. On any blood count showing 8.5 grams of hemoglobin or less.

5. On any blood count showing more than 5.5 million red cells or more than 16½ grams of hemoglobin.

6. On any leukocyte count above 20,000 or below 4,000.

7. On any leukocyte count above 12,000 showing more than 25% lymphocytes, both the total and differential leukocytes are repeated.

8. On any leukocyte count between 5-10,000 showing more than 80% neutrophiles, both the total and differential counts are repeated.

9. Rechecks are done on all counts showing abnormal blood cells in either the erythrocytic or leukocytic series.

When the term "repeat" is used, it means to do the count again, with another pipette.

All blood counting apparatus should be checked for accuracy. Only those pipettes which are guaranteed or which have correc-

tion factors should be used. No chipped or faulty pipettes should ever be used.

Moreover, it is well for the medical technologist to remember that frequently a physician's diagnosis is no better than his laboratory work; this is a challenge for the technician.

This discussion is based upon the fact that a blood count and syphilis tests are routine procedures, and the fact that oxalated venous blood is practical for all routine work in that it enhances the possibilities for a correct hematological diagnosis and reduces the number of skin punctures on a patient. The evidence against the use of ear-lobe blood for blood counts is increasing yearly. Counts may show discrepancies up to 17%.

When the ammonium-potassium oxalate mixture is used for an anti-coagulant, a definite amount must be used, 2mg per ml. Heat must be avoided when the containers are prepared. If the tubes are placed in the incubator, they will dry in a few days.

The needle and syringe must be dry, stasis avoided, and a clean cut puncture made. Needles used for collection should be at least 22 gauge or larger, preferably 20. Veni-puncture is easy if the equipment is in good condition.

The sample must be thoroughly admixed at the time of collection and before making dilutions for counts. This is frequently a great source of error in venous hematology.

Slides are made from a drop of blood from the needle, immediately after veni-puncture.

Up to date mechanical equipment such as shakers, tabulators, and binocular microscopes are essential to accurate work. Photometric estimations of hemaglobin and erythrocyte counts are not only accurate but time saving innovations for the laboratory doing a large volume of routine work. Hemocytometer counts are indicated on those cases showing abnormality.

It is the responsibility of the physician to recognize the various blood anomalies, but it is the technician's duty to present all salient details in a manner which bids for further studies when indicated. Anemia to polycythemia challenges correct differentiation—thus it is that hematology becomes a fixed science.

## THE MEDICAL TECHNOLOGIST AND DIABETES

SARAH ALLENE WISE, M.T. (ASCP), *Denver, Colorado*

The following is a study of the role that the medical technologist takes in an office in aiding the physician with the control of diabetes. The report is written under the guidance of E. Paul Sheridan, M. D. of Denver, Colorado.

Since the increasing incidence, the longevity, and the complications of diabetes have accentuated the problems of the medical technologist, her call of duty extends far beyond routine laboratory determinations. Hers should be a deep, accurate knowledge

of all phases of the disease. These laboratory procedures which are run more or less routinely will take on added importance as this responsibility becomes apparent.

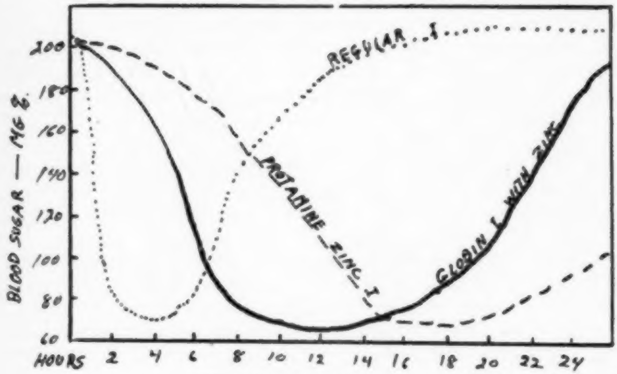
Of the laboratory tests, one most important is the blood sugar determination. The procedures are quite familiar to every medical technologist, but there are a few important influencing factors that I have found which increase the value of the determination to the diabetic and to the physician.

First, consider the influence that the anticoagulant has on the sugar content of the blood. A blood taken in any ordinary anticoagulant such as potassium oxalate or Wintrobe's anticoagulant, will not preserve the sugar content and a marked drop of nearly 10 mg% can be noticed within one hour after the blood has been drawn. Therefore, to have an accurate result, from the sugar determination, it should be run immediately if the ordinarily prepared tube is used. In preparing tubes for the doctor's use on house calls, one should note the fact that because they are not stable a preservative of sodium fluoride and monochlorbenzene, if not over-heated, will stabilize the sugar values up to four days duration. The formula for the preservative is in most textbooks; these sodium fluoride tubes are easily prepared and make for greater accuracy in your determination.

The next important influencing factor on the sugar value is time. A study of the glucose tolerance curve will show that a non-diabetic's blood sugar content will rise very quickly and always rapidly fall to normal. However, a diabetic's tolerance curve will rise above the normal limits and will remain high for several hours. Insulin does not correct this condition; so if a sugar is taken on a diabetic on P. Z. I. four hours after a meal, it will be higher than the non-diabetic at the same time. The reason for this is that the diabetic consumes his insulin for a day in one injection; at meal time, his pancreas cannot excrete enough insulin to burn all the carbohydrate consumed at that particular time, and so the increased sugar content appears in the blood. It is very important to the doctor to know how high a blood sugar content is at different definite times of the day corresponding to the type of insulin the patient is taking. "Why take a blood sugar at midnight, at 7:00 PM, or 11:30 AM," is often asked by technologists and is answered by the fact that different insulins give different effects.

To further emphasize the importance of blood sugar and time, a short review of the action of different insulins will be of value. Regular insulin acts in 2 to 6 hours, and protamine zinc insulin reaches its peak of action in 16 to 36 hours, and globin insulin has a peak of action about 8 to 12 hours after administration. The following chart will demonstrate this action of the different

insulins on the blood sugars, assuming the insulin is given at 7:00 AM.




(Chart is through the courtesy of E. Paul Sheridan, M.D.)

Studying the chart we find that if a fasting blood sugar is taken in the morning with the patient on regular insulin, that sugar will be very high. However, a determination taken about three hours after breakfast will be very low. Again it will rise before noon and fall about 3:00 in the afternoon, if insulin is taken again at 12:00. This shows that regular insulin is fast in action and of short duration. Now consider a person on protamine zinc insulin; he would find his lowest sugar content to be between midnight and breakfast. It is slower acting insulin of long duration. A globin insulin patient would find a sugar content of a high value in a fasting specimen, but about 3:00 to 5:00 in the afternoon he would expect a low sugar content. These hours are suggested as relative because each person is an individual case. The technologist should record the time and the blood sugar, and by doing so the insulin action on a specific patient can be determined by the doctor.

By disregarding the figures in the last row of the following example of a typical laboratory sheet as recorded by the medical technologist, we can illustrate the importance of time.

| | | | | |
|---|---|---|---|---|
| 9-10-48 | 9-10-46 | 5-11-47 | 5-11-47 | 8-1-48 |
| 368 mg% | 120 mg% | 285 mg% | 63 mg% | 94 mg% |
| fast. | 9:00 AM | 6:00 PM | 11:00 AM | 10:30 AM |

Notice how variable the quantities. At a glance one would assume an error in technique is present. However, such is not true. The apparent error is due to the absence of time recording and insulin type (regular in this case). Now by adding the hour

of determination, the values become significant to the clinician who is familiar with the case.

The fasting sugar is usually more constant than any other, however there are a few "brittle" diabetics who have undetermined influencing factors, in other words these are patients who cannot be classified by any set pattern. Such patients truly test the technologist's equipment, and technique, and evaluate the procedure.

The technologist must be cognizant of the blood source which greatly influences the value of the blood sugar. Venous blood is the usual source, but occasionally, especially on children, a micro-determination using a finger puncture may be adequate. This capillary blood being part of the arteriole system carries the balance. Remembering this, one will see readily that a capillary blood sugar taken after a meal might vary from the venous blood taken at the same time. It has been demonstrated that such a variation of 20-50 mg% is not uncommon; but a fasting micro-determination will not vary markedly. Dr. Herman O. Mosenthal has brought out this variation between capillary and venous values in his article "Interpretation of Blood Sugar" for the American Diabetic Association, 1945.

Besides the blood sugar determination, another important test a qualified technologist must perform to adequately ascertain diabetic control procedure is, first, the carbon dioxide combining power. This test is determined usually at a hospital in cases of acidosis. It is of value in ascertaining the degree of severity of the acidosis.

Second, the sodium, potassium, and chloride determinations must be made in cases of prolonged acidosis, because the salt exchange in the body due to marked dehydration has to be adjusted. The maintenance of this balance will necessitate rapid and accurate blood and urine determinations of the sodium, potassium, and chloride salts. I hope that very soon every medical technologist will have simple procedures for the determination of these salt values.

Third, in severe cases of diabetes, kidney damage complicates the condition. Kidney damage shows a change in the serum protein and non-protein-nitrogen values. In these cases these two determinations are as important as the blood sugar.

Last, the cholesterol value is also of significance in indicating the type of therapy to be employed especially in children's cases. The vascular change is frequently preceded by a change in the cholesterol value. As the procedures for its determination are quite varied, it becomes necessary to frequently check the method used, especially if a high cholesterol value is obtained.

The influencing factors on the tests just mentioned are only a part of the essential knowledge and understanding on the part

of the technologist to be of assistance in the control of the diabetic through the laboratory. Further technical determinations through urinalysis is of absolute importance to the physician. Beginning with the urinalysis for sugar, one must remember that the patient is also running tests at home. His home tests must agree with the laboratory and a standardization of colors and percentages should be established and checked frequently. In the urine sugar tests the influence of time, again, plays a very important role. A diabetic's urine test will show some sugar following a meal. At this time they spill their excess sugar. This knowledge is particularly helpful in taking a specimen for the detection of diabetes, as a fasting specimen may remain negative, and a non-fasting one show glucose long before the first symptoms of diabetes appears. Looking again at the relationship curve of blood sugar and insulin, one finds the time that sugar will most likely be found in the urine. However, a word of caution, glucose from a meal frequently remains in the bladder giving a positive test when the patient is having a reaction. Therefore the doctor does not depend entirely on the urine sugar results and requests a check by means of the blood sugar analysis.

Another factor to observe is that substances other than dextrose frequently will give a slightly positive reaction. Among these are asparagus and milk, when eaten in large quantities.

One of the questions which arises when a positive urine sugar is noted and one the physician usually asks is the value of the patient's threshold. As far as our tests have determined there is no definite threshold. Our patients range from 100 mg% to 250 mg% blood sugar before any spillover is seen. Normally the average of 180-200 blood sugar is accepted as the threshold.

Acidosis can be avoided by early detection of acetone. Many of the diabetics run their own acetone tests when their urine sugar tests have been high. This is of special value for children and pregnant diabetics because they drop into an acidosis very rapidly.

This brings to mind tests for other complications of diabetes such as kidney damage and pregnancy. Albuminuria is one of the first indications of kidney damage; but a high protein diet will occasionally cause a trace of albuminuria and contamination will give false positives. False positives should be rechecked because they are definite danger signs to the physician concerning his patient.

Recently the use of hormones in the treatment of pregnancy of diabetics initiated the determination of pregnandiol in the urine. Pregnandiol is the excretion product of the breakdown of these hormones. Dr. Priscilla White of Boston is doing extensive work with the relationship of pregnandiol and hormone

therapy in diabetic pregnancies. She has proven unquestionably the value of the hormonal therapy for this condition. The laboratory procedure for pregnandiol determination as yet is far too complicated for the office laboratory, but soon it should be simplified for it is one of the most important guides to the therapy necessary for pregnant diabetic women.

The duties of the medical technologist to the clinician who cares for diabetics does not stop with the recording of the result of the laboratory determinations. It includes the responsibility of noting and recording the factors which influence the value of these determinations And it also includes the knowledge and practice of "diabetic first aid" in emergencies.

This latter phase of her duty is very important in the small office where the patient is cared for and educated. "First aid" for diabetics is educated care in diabetes. Knowledge of "diabetic first aid" is not for the patient alone, but for everyone who cares for a diabetic. The diabetic lives with his disease his entire life. He is the one who feels the reaction, who feels the coma, who tests his urine, and who is most interested in his case. He is entitled to know as much about it as he can. The education of the diabetic and his family was formerly the job of the physician, but with the increased number of diabetics it is virtually impossible for him to give detailed instruction to each patient. Dr. Joslin has stated in his "Introduction to Symposium on Diabetes" that the physician must make "special provisions for their [diabetic's] education. This will require so much time that they must have assistance . . ." This is where we can assist as medical technologists.

By education is meant the history of diabetes, health, injuries, diet, insulin, syringes, blood sugar, urinalysis, reactions, comas, and dangers. The subject is large but it is the basis of "diabetic first aid." "First aid" for diabetics can best be illustrated by demonstrating a case. Miss B's sister called that Miss B was vomiting and acting "funny." When she called she wanted immediate help. If she had been educated in "diabetic first aid," she would have known what to do until the doctor arrived. A few history questions were asked such as the time and method of onset of the illness; and whether the patient had had her breakfast and insulin. Because of her education in diabetic care, the technologist gave her a few reassuring answers such as a reaction comes on suddenly within two hours; a patient who has taken insulin but has had no breakfast is liable to a reaction; and that comas usually do not occur as long as a test is blue. The one of the first rules of "diabetic first aid" is given—when in doubt give sugar. The other rule which must be adhered to by all of us is never give insulin unless prescribed by the physician. The patient gains an increased confidence in the

technologist as well as herself.

Another demonstration of the service that a technologist may render is giving to a substitute doctor any information which might be of value to him in caring for the unfamiliar patient. An illustration of this is the case of Mr. L. His blood sugar was kept very low with a constant threat of a reaction. The doctor was called away, and at that time Mr. L went into a very severe reaction. The doctor on call phoned for a quick summary of the case and was informed that the family had been cautioned that in case of a reaction the patient must have salt as well as sugar. The salt was very important to this patient as he was on a rice diet for kidney failure. Other incidents paralleling this can be quoted to show that a responsibility is placed on the technologist beyond that of laboratory reports.

In summation I trust I have made it clear that the duties of the technologist are of great importance to the physician who is controlling diabetics. The office laboratory responsibilities require that the technologist must be adequately trained, observant, conscientious, and throughly educated in "diabetic first aid."

**BIBLIOGRAPHY**

Allyn, R. "Why Is Blood Carbon Dioxide Valuable in Diabetes?" *American Journal of Medical Technology*. **13:** 113-116. May, 1947.

Goldzicher, Joseph W. "New Colorimetric Method for Determination of Pregnandiol." *Journal of Laboratory and Clinical Medicine*. **33:** 251, February, 1948.

Joslin, Elliott P. "Introduction to Symposium on Diabetes." *Medical Clinics of North America*. Nationwide number, 1947.

Joslin, Elliott P. *Diabetic Manual*. Edition 8, Philadelphia: Lea & Febiger, 1948.

Mosenthal, Herman O., and Barry, Ellen. "Interpretation of Blood Sugars." *Proceedings of the American Diabetes Association*. **5:** 141-162.

Todd, James C., and Sanford, Arthur H. *Clinical Diagnosis*. Edition 10. Philadelphia and London: W. B. Saunders Company, 1944.

White, Priscilla. "Pregnancy Complicating Diabetes." *Pennsylvania Medical Journal*. **50:** 705-708. April, 1947.

## MEDICAL BACTERIOLOGY IN THE SMALL LABORATORY USING COMMERCIAL DEHYDRATED AND PREPARED MEDIA

By DOROTHY A. FOREMAN, B.S., M.T. (ASCP)

To give complete coverage in medical bacteriology in the small laboratory often seems an impossibility. Limited time and facilities make the time-consuming service a neglected science in many laboratories. The purpose of this paper is to point out that with the use of available dehydrated and prepared culture media and simplified identification procedure, the required types of bacteriologic examinations can be made in the small laboratory.

The method of culturing, choice of media and staining methods should be adapted to the preference of the medical technologist and the type of work to be done. Those presented are for the purpose of illustration and are not necessarily the best method of identification.

In addition to the usual microscope, glassware and pipettes found in clinical laboratories, special equipment required consists of an incubator, refrigeration and autoclaving facilities. A variety of models of autoclaves are available,[6] ranging from large hospital types (which may also serve the laboratory) to the portable type, as the pressure cooker. It is recommended that glassware be sterilized by dry heat,[4] but if a hot-air sterilizer or oven facilities are not available, autoclaving may be used.[5] Pipettes and Petri dishes may be wrapped with paper, if special cans are not provided. A 2-pound coffee can with a tight-fitting lid makes an excellent Petri dish container.

Many authorities[4,5] suggest the use of dehydrated media in small laboratories. Prepared tubes of media may be purchased at a nominal cost for occasional cultures. Dehydrated media may be purchased in small quantities, a few well-selected types furnishing culture material for a wide variety of organisms.

The use of several special staining procedures are definite aids in the identification of bacteria. Methods may be selected that use the solutions of Gram's and Ziehl-Neelsen stains, thereby eliminating the deterioration and waste of little-used special stains. Loeffler's alkaline methylene blue solution (also used in Ziehl-Neelsen stain) may be used for morphology and metachromatic granules of *Corynebacterium diphtheriae*, allowing the stain to remain on the slide for ten minutes.[16] The Hiss method of staining capsules,[21] using Gram's crystal violet or Ziehl-Neelsen carbol-fuchsin requires little time. A spore stain, as Dorner's method,[3] which uses carbol-fuchsin and Loeffler's methylene blue, may be of occasional value.

For culturing staphylococci, streptococci and pneumococci, a variety of dehydrated and prepared media is available. Dehydrated media, as brain heart infusion, dextrose broth and agar, tryptose phosphate broth and many others, suitable for the above organisms, require little time in preparation. These may be obtained from Difco Laboratories, Inc., Detroit, Michigan, and the Baltimore Biological Laboratories (BBL). Baltimore, Maryland. Literature is available from these companies giving excellent descriptions of their products.[7,8,9,10] Prepared tubes of bouillon, gelatin, nutrient agar and others may be obtained from Difco. Also, litmus milk and Ulrich milk (contains two indicators) may be purchased from the same source. Blood agar, a necessary media in the culturing and identification of many organisms, may be supplied by preparation from dehydrated blood agar base, Difco or BBL. This media requires only dissolving and sterilizing and a quantity of proper size flasks may be kept on hand and plates poured as needed. Sources of blood may be rabbit or sheep blood, surplus sterile blood collected from various tests, blood bank or defibrinated placental blood as rec-

ommended by Fitzgerald.[12] The blood agar base may be used without the addition of blood as a slightly acid agar. For quick or occasional cultures, prepared blood agar tubes may be kept on hand, obtainable from Difco Laboratories.

For *C. diphtheriae* cultures, in addition to blood agar, Loeffler's blood serum agar may be purchased from Difco and Lederle Laboratories[15] in prepared tubes. Also, chocolate tellurite agar tubes are available from Difco. These prepared tubes are ready for use, inexpensive and keep well.

For blood cultures, several media are recommended, all easy to prepare and preserve. Kracke blood culture media, tryptose phosphate broth or brain heart infusing may be used. If blood culture bottles are not provided, ordinary 8-ounce, small mouth baby bottles with rubber bottle caps may be used. The media may be sterilized in the bottle with a cotton plug, to be replaced by a sterile rubber cap when cooled. Blood may be added to the media by inserting the syringe needle through the rubber after sterilizing with an alcohol sponge. A blood culture outfit, recommended for isolation of organisms except strict anaerobes, that is ready for immediate use may be obtained from Lederle Laboratories, Kansas City, Missouri (branch office in this territory). A simplified technic for cultures for enteric organisms from the blood clot, employing a 3% glucose-bile medium is reported by Rappaport.[19] Suitable culture media as recommended[18] may be selected for specific organisms.

A variety of media, prepared and dehydrated, is available for culturing organisms of the Neisseria group. The use of Difco Proteose No. 3 agar, hemoglobin and supplements[10] prove quite satisfactory. Recommended by the Baltimore Biological Laboratories for Neisseria and other fastidious organisms, cystine trypticase agar may serve as a medium for maintenance, identification, motility and, with added carbohydrates, for the determination of fermentation reactions. From a comparative study by Weller and Williams,[22] Difco dehydrated Mueller-Hinton starch agar seems most practical. A simple medium for the isolation of gonococcus, prepared from egg yolk and proteose No. 3 agar (Difco), is reported by Hirschberg.[14] This media is described as easily prepared, therefore is particularly useful for small laboratories. For occasional cultures, prepared tubes of chocolate agar, glucose ascites agar, ascitic agar and other are supplied by Difco Laboratories.

Culturing of *Mycobacterium tuberculosis* is simplified by the purchase of Dorset egg medium, Petroff egg medium or Petragnani medium in tubes prepared by Difco.

The enteric organisms may be isolated using dehydrated media as Wilson-Blair bismuth sulfiate agar, S. S. agar, MacConkey agar, Eosin methylene blue agar, Endo agar, sodium desoxycho-

late-citrate agar and others. All are easy to prepare and the use of several, as Wilson-Blair, S. S. and MacConkey's, insure good results. For further identification, media are available that serve several purposes in one culture, as T. S. I. (triple sugar iron) agar by Difco and BBL designed by Hajna,[13] the one medium indicating the ability of an organism to ferment lactose, sucrose and dextrose with the formation of acid and gas, and also, its ability to produce hydrogen sulfide. SIM medium (Difco) is a combination medium showing hydrogen sulfide and indol production and motility in the same tube. A presumptive identification may be made on motility, indol production, hydrogen sulfide production, acid and gas formation, and fermentation of lactose, sucrose and dextrose. If further fermentation studies are required, phenol red broth or agar with the more frequently used carbohydrates are obtainable in dehydrated form. Other aids in identification may be employed, as urease reaction and gelatin liquefaction, using dehydrated media. Serological identification may be made using anti-typhoid, anti-paratyphoid A & B, polyvalent Salmonella and polyvalent Shigella anti-serum from Lederle Laboratories. The use of polyvalent serum is suggested to give coverage with a small supply of reagents. A method of agglutination identification requiring a single serum dilution and incubation technic requiring a matter of minutes is presented by Feldman.[11]

For culturing anaerobes several simple methods are suggested by Bryan.[1] Also, the "shake culture"[2] in which the semisolid media are boiled to drive out the oxygen, cooled to 42 degrees and innoculated. The Spray dish which uses pyrogallic acid and an alkali to absorb the oxygen may be used for plate cultures. Rosenthal's anaerobic method as described by Zinsser and Bayne-Jones[6] may be carried out in a Mason fruit jar. Dehydrated media for anaerobes are available from Baltimore Biological Laboratories[8] and Difco. Fluid thioglycollate medium is suggested for the "aerobic" cultivation of anaerobes.

If animal inoculations are desired, single cages may be purchased for maintaining a few animals.

For organisms requiring special media, dehydrated and some prepared media are available, as Bordet-Gengou agar base for *Hemophilus pertussis,* cystine heart agar for *Pasteurella tularensis* and tryptose agar for Brucella organisms.

Media for yeasts and molds are available in dehydrated form and some in prepared tubes, including Sabouraud dextrose agar, Sabourand maltose agar, Littman Oxgall agar, and others supplied by Difco and Baltimore Biological Laboratories.

Dehydrated media are prepared especially for the assay of the potency of antibotic, conforming to the specifications prescribed by the Food and Drug Administration in the Federal Register.[7,9]

With little time and effort, a number of differential characteristics may be observed. Inulin fermentation and bile solubility for differentiating pneumococcus and streptococcus, coagulase test for differentiating pathogenic and non-pathogenic staphlococci,[16,17] methyl red and Voges-Proskauer to differentiate *Escherichia coli* and *Aerobacter aerogenes,* liquefaction of gelatin and other aids as the use of sodium azide in isolating Gram positive cocci from Gram negative bacilli as described by Schwarting[20] are a few identification tests that may be used.

A careful selection of media will result in a small supply which will be adaptable to the cultures required. The use of commercially prepared media offers the advantages of uniformity, dependability, economy and convenience. Other sources of media are probably available, but are not mentioned in the literature on hand.

## Summary

Bacteriologic examinations in the small laboratory may be practical through the use of (1) special staining methods that use general staining solutions, (2) a minimum of equipment, in addition to available laboratory facilities and (3) commercial dehydrated and prepared culture media.

## REFERENCES

### Books

1. Bryan, Arthur H. and Bryan, Charles G. Principles and Practice of Bacteriology, Third Edition. New York; Barnes and Noble, 1942,
2. Jordan, Edwin O. and Burrows, William. Textbook of Bacteriology thirteenth Edition. Philadelphia: W. B. Saunders Company, 1941.
3. Kolmer, John A. Clinical Diagnosis by Laboratory Examinations. First Edition. New York: D. Appleton-Century Company, 1944.
4. Kolmer, John A. and Boerner, Fred. Approved Laboratory Technic, Fourth Edition, New York: D. Appleton-Century Company, 1945.
5. Stitt, E. R. Clough, Paul W. and Mildred C. Practical Bacteriology, Hematology and Animal Parasitology, Ninth Edition. Philadelphia: The Blakiston Company, 1944.
6. Zinsser, Hans and Bayne-Jones, Stanhope. A Textbook of Bacteriology. Eighth Edition, New York: D. Appleton-Century, 1939.

### Manuals, Periodicals and Pamphlets

7. Baltimore Biological Laboratories, Baltimore, Maryland. Products for the Bacteriological Laboratory and Powdered Culture Media and Reagents.
8. Baltimore Biological Laboratories, Baltimore, Maryland. Culture Media for Clostridia and other Anaerobes.
9. Difco Laboratories, Inc., Detroit, Michigan. Manual of Dehydrated Culture Media and Reagents. Eighth Edition.
10. Difco Laboratories, Inc., Detroit, Michigan. Diagnosis of Gonococcal Infections by the Cultural Methods.
11. Feldman, Harry A.: Centrifuged Reactions for Serum Diagnosis and Bacterial Identification. Tech. Bul. of Reg. of Med. Tech. 7:25, 1946.
12. Fitzgerald, John T.: A Source of Blood for Culture Media. Am. Jour. Med. Tech. 11:272, 1945.

13. Hajna, A. A.: Triple-sugar Iron Agar Medium for the Identification of the Intestinal Group of Bacteria. Jour. Bact. 49:516. 1945.
14. Hirschberg, Nell. North Carolina State Laboratory of Hygiene, Rawleigh, N. C. Reported in Central Electric X-Ray News. Vol. 19, No. 12.
15. Lederle Laboratories, Man Office, New York. Diagnostic Reagents.
16. Marsh, Homer F. A Laboratory Guide for Bacteriology and Serology. University of Oklahoma School of Medicine, Oklahoma City, Okla.
17. Miale, John B. and Frye, J. W.: Standard Test for Staphylococcus Coagulase Activity. Tech. Bul. Reg. Med. Tech. 18:95, 1948.
18. Naval Medical School Laboratory Guide, Bacteriology. National Naval Medical Center, Betheseda, Maryland.
19. Rappaport, F.: Simplified Technic for Culture from Blood of Enteric Groups of Bacteris. Tech. Bul. Reg. Tech. 8-17:904, 1947.
20. Schwarting, Virginia M.: Use of Sodium Azide in Isolation of Gram-Positive Cocci. Tech. Bul. Reg. Med. Tech. 7:123, 1947.
21. War Department. Methods for Laboratory Technicians, Technical Manual, 8-227, Washington, D. C., 1941.
22. Weller Thomas H. & Williams, John E.: Comparative Study of Media for the Primary Isolation of the Gonococcus. Amer. Jour. Syph. Gonor. and Ven. Dis. Vol. 30, p. 386, July, 1946.

# DERIVATION OF THE CORPUSCULAR CONSTANTS

WILLIAM R. BEST, M.D. and LOUIS R. LIMARZI, M.D.

Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration are clinical determinations employed extensively and to good purpose. Though most clinicians and technicians appreciate their significance, few have calculated their derivation from macro to micro unitage. A perusal of such derivation is of value to the physician so that he may better appreciate the meaning of these concepts and have confidence in their standard formulae.

Wintrobe[5] originated and popularized these constants expressing them respectively in cubic microns, micromicrograms, and per cent. Prior to that time Hayden[2] had performed similar calculations designating the individual cell volume in cubic centimeters times $10^{-11}$, the individual cell hemoglobin in grams times $10^{-11}$, and the hemoglobin saturation in per cent. Through less accurate methods these values were approximated even before that time. Thus, Welcker[4] in 1863 calculated the cell volume in "cubic micromillimeters" from the average cell diameter and thickness (average normal = 72.2). Malassez[3] calculated the "titre hemoglobinique" in micromicrograms as early as 1873. Widespread application awaited the development of more practical clinical methods and a wider appreciation of the diagnostic aid afforded by these constants.

In none of the original papers nor in any of the standard texts of hematology or clinical pathology is the step-by-step derivation of final units discussed. Therefore, we have placed the successive

---

From the Department of Internal Medicine, University of Illinois College of Medicine, Chicago, Illinois. Aided by a grant from the Hematology Research Foundation.

stages of derivation for these constants into as clear and simple a format as possible.

*Derivation of Mean Corpuscular Volume*

According to the formulae given in the texts, mean corpuscular volume *(MCV)* in cubic microns is equal to the number of cubic centimeters of packed cells per 1000 cc. of whole blood divided by the red blood cell count *(Rbc)* in millions per cubic millimeter:

$$MCV \text{ (cubic microns)} = \frac{\text{vol. packed cells, cc. per 1000 cc. whole blood}}{Rbc\text{, millions per c.mm.}}$$

To obtain the volume of the mean cell, one must divide the volume of a sample of packed cells (i.e. that of 1000 cc. whole blood) by the total number of red cells in that sample (i.e. the number in 1000 cc. of whole blood). As *Rbc* represents the number of red cells in millions per cubic millimeter, the true number of cells per cubic millimeter must equal $Rbc \times 10^6$. There are $10^3$ cubic millimeters per cubic centimeter and $10^3$ cubic centimeters in the 1000 cc. sample. Hence, the total number of red cells in that 1000 cc. sample equals $Rbc \times 10^6 \times 10^3 \times 10^3 = Rbc \times 10^{12}$.

$$\text{Thus: } MCV \text{ (cc.)} = \frac{\text{vol. packed cells, cc. per 1000 cc. whole blood}}{Rbc \text{ (millions per c.mm.)} \times 10^{12}}$$

1 cc. $= 10^{12}$ cubic microns. (1 cc. $= 10^3$ c.mm.; 1 c.mm. $= 10^9$ cubic microns)

$$\text{Therefore: } MCV \text{ (cubic microns)} = \frac{\text{vol. packed cells, cc./1000 cc.} \times 10^{12}}{Rbc \times 10^{12}}$$

$$= \frac{\text{vol. packed cells, cc./1000 cc.}}{Rbc}$$

*Derivation of Mean Corpuscular Hemoglobin:*

Mean Corpuscular Hemoglobin (MCH) is given as the grams of hemoglobin in 1000 cc. of whole blood divided by *Rbc* in millions per c.mm.:

$$MCH \text{ (micromicrograms)} = \frac{\text{hemoglobin, gm. per 1000 cc.}}{Rbc\text{, millions per c.mm.}}$$

The weight of hemoglobin per mean cell is obtained by dividing the weight of hemoglobin in a given sample of blood (i.e. 1000 cc.) by the number of cells in that sample. As for the *MCV* the total number of cells in 1000 cc. of blood $= Rbc \times 10^{12}$.

$$\text{Thus: } MCH \text{ (grams)} = \frac{\text{hemoglobin, gm. per 1000 cc.}}{Rbc \times 10^{12}}$$

1 gram $= 10^{12}$ micromicrograms.

$$\text{Therefore: } MCH \text{ (micromicrograms)} = \frac{\text{hemoglobin, gm. per 1000 cc.} \times 10^{12}}{Rbc \times 10^{12}}$$

$$= \frac{\text{hemoglobin, gm. per 1000 cc.}}{Rbc}$$

*Derivation of Mean Corpuscular Hemoglobin Concentration:*

Mean Corpuscular Hemoglobin Concentration *(MCHC)* is given in the texts as the weight of hemoglobin in grams per 100 cc. of

whole bloods times one hundred divided by the volume of packed cells in cc. per 100 cc. of whole blood:

$$MCHC \text{ (per cent)} = \frac{\text{hemoglobin, gm. per 100 cc.} \times 100}{\text{vol. packed cells, cc. per 100cc.}}$$

As stated, the result is expressed in per cent. Mathematically, this is not an accurate term to use, but it is the most convenient one available and is well established by usage. Actually *MCHC* expresses the weight of hemoglobin in a given volume of packed red cells. It reflects the hemoglobin saturation of the cell regardless of the cell size.

$$MCHC \text{ (Grams Hbg. per cc. packed cells)} = \frac{\text{hemoglobin, gm./100 cc.}}{\text{vol. packed cells, cc./100 cc.}}$$

The unit, "grams hemoglobin per cc. packed cells," is awkward, and the numerical result would be expressed as an inconvenient decimal. If one assumes that 100% concentration is equal to 1 gram of hemoglobin per 1 cc. of packed cells, he merely multiplies by one hundred to convert this quotient into per cent.

$$\text{Thus: } MCHC \text{ (per cent)} = \frac{\text{hemoglobin, gm. per 100 cc.} \times 100}{\text{volume packed cells, cc. per 100 cc.}}$$

Actually this value never reaches 100%. Thirty-eight per cent is the highest saturation observed clinically.

### *Clinical Range of Values*

Having derived the formulae above, the rationale for unitage is obvious. In clinical practice the range of values is such that one may perform the problems of division without regard for decimal points. The decimal point falls into its natural place according to numerical value.

*e.g.* Extreme ranges of *MCV* are 50 to 160 cubic microns. Thus, in a clinical problem resulting in the figures 1087, it is inconceivable that the *MCV* would be 10.87 or 1087.0. The only reasonable figure is 108.7 which would indicate a macrocytosis. The clinical range for *MCH* is 15-56 micromicrograms and for *MCHC* is 15-38%.

These calculations are readily carried out by slide rule, nomogram, or by means of special hematologic calculator.[1]

One must be extremely cautious to insert the correct numerator and denominator in each calculation. If these be inverted, they will often result in false values which fall within the usual clinical range.

*e.g.* With $Rbc = 5.0$ million and hematocrit $= 45\%$ (disregarding decimal points)
Hematocrit/$Rbc = 45/5 = 90$ (correct)

*Rbc*/Hematocrit = 5/45 = 111 (incorrect)
These two values carry different clinical significance but both fall within the clinically observed range.

## Summary

The derivation of unitage for the three corpuscular constants is traced, and a few practical comments regarding their clinical application are brought forward.

## BIBLIOGRAPHY

1. Best, W. R.: A Hematologic Slide Rule for Calculating the Corpuscular Constants, Jour. Lab. and Clin. Med., **34**: 434-437, 1949.
2. Haden, R. L.: Accurate Criteria for Differentiating Anemias, Arch. Int. Med. **31**: 765-782, 1923.
3. Malassez, L. C.: De la númeration des globules rouges du sang, Paris, A. Delahaye, 1873.
4. Welcker, H.: Grösse, Zahl, Volum, Oberfläche und Farbe der Blutkörperchen bei Menschen und bei Thieren. Zeitschrift für Rationelle Medicin, 3d. ser., **20**: 257-307, 1863.
5. Wintrobe, M. M.: Classification of Anemias on Basis of Differences in Size and Hemoglobin Content of the Red Corpuscles, Proc. Soc. Exper. Biol. and Med., **27**: 1071-1073, 1930.

---

Note: Thanks are due to M. M. Wintrobe for helpful suggestions.

# BOOK REVIEW

**BACTERIOLOGICAL TECHNIQUE. A guide for Medical Laboratory Technicians by W. W. W. McEwen, A.I.M.L.T., F.R.M.S. Chief Technician, Pathological Laboratory, Neath General Hospital, Late Chief Technician and Lecturer in Bacteriology, Nottingham and District Technical College, Nottingham with a Foreword by Professor Sir Alexander Fleming, F.R.C.P., F.R.C.S., F.R.S. 293 pages, 70 illustrations. London J. & A. Churchill Ltd., 1949. Price not given.**

This book is written by an English medical laboratory technician who has had a vast experience. The language of the text is simple; the author's style direct, the subject matter exclusively inclusive. Before leaving the first page of the first chapter, one is convinced that this is a small volume of great value with a wealth of information that can be obtained from only those with "know how." In regard to the linoleum flooring one reads "Continual walking will cause linoleum to stretch slightly when it will act rather like a pair of bellows—each time a weight is put on it the dust underneath will be blown out into the atmosphere of the room."

There are thirty one chapters in this handbook entitled as follows: Laboratory Accommodation and Equipment, Care of Laboratory Glassware and Equipment; Preparing Routine Glassware and Apparatus for Sterilization; Special Apparatus for Collection of Specimens; Laboratory Glassblowing; Upkeep of Laboratory Stock; Laboratory Records of Specimens; Labora-

tory Animals; Animal Post-mortem Technique; Sterility and Sterilization; Centrifuges; Immunity and Immunological Technique; Wassermann, Kahn and Similar Reactions; General Culture Technique; Anerobic Culture Technique; Stock Cultures; Preparation of Culture Media; Bacteriological Filters; Sections; Chapters on the Routine Examination of water, milk, shell fish, stools, swabs, sputa, etc., Slide preparations for Microscopical Examinations; Stains, Staining Methods and fixatives, and The Microscope.

The chapters on Animals and Post-mortem technique are very valuable in that so few texts give proper attention to these two very important subjects in the practice of every day bacteriology.

In passing the reviewer wishes to emphasize that this book should be on the reference shelf of all bacteriology laboratories, especially those training students. Although it is not comprehensive and does not contain references, the material submitted has withstood the test of time; many secrets of success and failure have been included in this timely small volume.

## ABSTRACTS

**POLYVINYL ALCOHOL-FIXATIVE AS A PRESERVATIVE AND ADHESIVE FOR PROTOZOA IN DYSENTERIC AND LIQUID MATERIAL: By M M. Brooke, DSc, and Morris Goldman, M.S. From the Laboratory Division, Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga. Jour. Lab. and Clin. Med. 34: 1554 (Nov.) 1949.**

The authors state that their article does not represent an endorsement of this product by the Public Health Service.

During the past decade the use of PVA has been described in the literature for use as a mounting medium for insects, fungi and tissues and, as an agent for reducing motility of paramecia and other small organisms.

The fixative is prepared as follows: 5 grams of powdered PVA (Elvanol 90-25 obtained from the E. I. Du Pont de Nemours and Company, Electrochemical Department, Wilmington 98, Delaware) were added to a mixture at room temperature containing 1.5 ml glycerol, 5 ml of glacial acetic acid, and 93.5 ml of Schaudinn's solution (2 parts of saturated aqueous solution of mercuric chloride to 1 part of 95% ethyl alcohol). Heat while stirring to approximately 75° C. A water-clear non-lumpy solution should be obtained and which remains satisfactory for several months.

On microscopic slides 3 drops of fixative are mixed with 1 drop of a specimen and the mixture smeared over 1/3 of the surface. Drying is accomplished by placing in the incubator over night. In vials the ratio of fixative to material is three parts or more. Staining is usually done by the long Haidenhain hemotoxlin method.

The authors feel that this technique has the advantage in that it permits the successful staining of organisms in liquid stool specimens, and also, that it offers a means for the preservation of interesting specimens for teaching purposes and subsequent staining.

Attention is called to the fact that PVA stains are not to be recommended for diagnosis of helminth ova and large cysts.

**RAPID METHOD OF PREPARING SCHIFF'S REAGENT FOR THE FEULGEN TEST: By Jerome Alexander, Kenneth S. McCarty, and Eleanor Alexander-Jackson. 50 East 41st St., New York City, N. Y. Science 111:13 (Jan. 6) 1950.**

To 100 ml of 0.5% fuchsin base, add 5 g active and undecomposed sodium hydrosulphite, and at the same time add 0.25 g of activated charcoal (Nuchar). Shake thoroughly in a closed flask; filter through coarse filter paper. The finished product is light amber.

The tissue section to be examined is hydrolyzed in N HCl for 4 min. at 60° C., washed, and allowed to stand in the leuco-base solution for 2 hr. Layer solution with xylol to prevent oxidation. Wash slides first in 0.25% sodium hydrosulphite, then in distilled water. Clear and mount.

**THE FATE OF PLASMA CELLS:** By P. Bueno, Department of Pathology, Instituto Biologico, Sao Paulo, Brazil. Science 111, 60 (Jan. 20) 1950.

Observations made in the course of a study of experimental C avitaminosis in the guinea pig revealed very clearly that plasma cells undergo transformation into reticular elements. Initially the plasma cells accumulated in great numbers in the medulla of the lymph nodes and showed transitional phases before assuming reticular cell cytology.

The author concludes that his findings confirm the assumption that plasma cells are not doomed to degeneration but that they are cells in a phase of resistance, still maintaining an evident capacity for proliferation and differentiation.

**A CLINICAL EVALUATION OF THE BLACK-KLEINER-BOLKER BLOOD TEST FOR MALIGNANT DISEASE:** By M. M. Stettnerm, M.S., H. Baron, M.S., B. E. Lowenstein, M.D., et al, from the Department of Physiology and Biochemistry, the Cancer Detection Clinic, the Department of Surgery, and the Department of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.

These authors state that "while this test is not entirely specific for cancer, the rather small number of false negatives has raised the possibility of using it as a screening test to indicate the patients whose further study is warranted to determine the possible presence of cancer."

Since the technic used is given in detail, the abstract author is including it for the perusal of its readers.

### Methods

*Sample:* 10 ml of fasting blood (Drugs such as penicillin, streptomycin, antihistaminics, and the sulfas interfere with the test) are withdrawn with a dry syringe and are placed in a bottle containing sufficient Potassium oxalate to prevent clotting. The contents are mixed by rotation; Centrifuge within one hour at 3000 rpm for 20 min. Pipette off plasma; save—place in refrigerator if not tested at once.

*Methylene Blue Reduction Time:* (practically verbatim) To 1 ml of plasma in a 10 x 100 ml tube exactly 0.2 ml of a standardized solution of methylene blue (150 mg/100 ml water) is added. Standardized methylene blue is defined as one which gives reduction times of 7-9 minutes with normal plasmas. One drop of capryl alcohol may be added to prevent foaming; the tube is immediately placed in a 400 ml beaker containing between 200-250 ml of vigorously boiling water. It is imperative that the water be boiling briskly, otherwise the temperature near the surface will fall significantly below 100° C., and reduction of the dye will not occur. Without removing the tube from the water bath, the time of disappearance of the last trace of color is recorded to the nearest half minute and noted as the methylene blue reduction time.

It is necessary to observe the disappearance of color without removing the tube from the water bath because the dye will immediately regain its color if cooled below 100° C.

*Heat Turbidity Index:* To 1 ml of plasma in a Klett colorimeter tube are added 4 ml of distilled water; mix. The tube is then placed in a Klett colorimeter using a 540 mu filter and the optical density is recorded. The tube is then placed in a bath of briskly boiling water, with a quantity sufficient to cover 9/10 of the tube. The tube is held in place for exactly 10 seconds; then removed and cooled immediately for 30 seconds in cold running water. The increase in optical density after heating is called the heat-turbidity index.

*Cautions:* Care must be taken to determine both the methylene blue reduction time and the heat turbidity index as soon as possible after the blood is drawn. Blood must be stored in the refrigerator unless tested immediately. No test should be performed if the sample is over 24 hrs. old. The plasma should be removed from the cells within one hour after drawing sample.

**A COLORIMETRIC METHOD FOR THE DETERMINATION OF CHLOROMYCETIN IN SERUM AND PLASMA:** By Samuel P. Bessman, M.D., and Sara Stevens, B.S. From Research Foundation of Children's Hospital, Washington, D. C. Jour. Lab. and Clin. Med. 35:129 (Jan.) 1950.

The authors describe a microcolorimetric method for the determination of chloromycetin in 1 ml samples of serum, based on the reduction of the aryl nitro group with stannous chloride, and the subsequent diazotization and coupling to produce a red complex.

MATERIALS AND METHODS. *Essentially verbatim*

**Reagents:**

*Barium Hydroxide,* (0.3 N appr.) Dissolve 15 Gm of anhydrous Barium Hydroxide in 500 ml hot water; cover, allow to cool. Filter into a bottle provided with a siphon outlet and a soda-lime tube for air inlet.

*Zinc Sulphate:* (5% appr.) Dissolve 25 Gm of $ZnSO_4 \cdot 6$ HOH in 500 ml of water. Adjust by titrating 5 ml of $ZnSO_4$ diluted with 20 ml of water against the Barium Hydroxide, using phenolphthalein as indicator, and dilute so that 5 ml of the Zinc Sulphate are neutralized by 4.7 to 4.8 ml of the Barium Hydroxide. The solution must be swirled vigorously after each addition of the Barium Hydroxide, and the end point a permanent pink.

*70% Hydrochloric Acid:* Dilute 70 ml conc HCl to 100 ml.

*Stannous Chloride:* 0.80 Gm/100 ml of 70% HCl—prepare fresh weekly.

*Sodium Nitrite:* 1% (one per cent) prepare weekly, store in refrigerator.

*Sulfamate Reagent:* 0.50 Gm Ammonium Sulfamate, 27.6 Gm $NaH_2PO_4 \cdot$ HOH made up to 100 ml.

*Coupling Reagent:* 0.20 Gm n-(1-naphthyl) ethylene diamine dihydrochlori per 100 ml. Store in brown bottle in refrigerator.

*Chloromycetin Standard:* 100 mg/100 ml. Stopper tightly; keep in refrigerator.

*Preparation of Filtrate:*

Place 5 ml water in centrifuge tube; add 1 ml serum.
Add 2 ml Barium Hydroxide; mix well.
Add 2 ml Zinc Sulphate solution—Allow to stand a few minutes.
Centrifuge appr. 2000 rpm for 10 minutes.

*Reduction:* Place 2 ml of the supernatant in each of two colorimeter tubes calibrated at 6 ml. Label T (test) and B (blank). Make a Reagent Blank.

To tube T add 0.5 ml of stannous chloride solution (0.80 Gm/100 ml 70% HCl).

To tube B add 0.5 ml 70% HCl.

Place in a boiling water bath for exactly 20 minutes. Remove and cool in cold water to room temperature.

*Color Development:* Add 0.5 ml of sodium nitrite to each tube; let stand 2 minutes.

Add 2.0 ml sulfamate Reagent to each tube, mix well by shaking; let stand 3 minutes.

Add 0.5 ml of coupling Reagent, dilute to 6 ml mark with water, let stand 20 minutes.

Read both tubes against the reagent black at 555 mu.

*Standard Curve:* Dilute some of stock chloromycetin 1-100 to give a working solution of 10 gamma/ml. 2 ml of this solution are equivalent to 2 ml of filtrate from sera containing 100 gamma of chloromycetin/ml.

A series of four dilutions of the 100 gamma standard are prepared by placing 2, 4, 6, and 8 ml of the working standard in 10 ml vol. flasks and bringing to mark with distilled water. These dilutions correspond to 20, 40, 60 and 80 gamma per ml sera respectively. 2 ml of each of these dilutions are placed in colorimeter tubes and a water blank is run with the set.

A standard of 20 gamma is equivalent to 100 gamma per ml and provides a factor for calculation. Readings are taken from the 2-log T scale:

$$(T - B) \times \frac{1}{St} \times 100 = \text{Gamma per ml of serum.}$$

## WHO WORKS FOR YOU?

There are so many interesting subjects embodied in that Constitution and By-Laws of ours that it's hard to choose just one to talk about now. Since it would be rather unwieldy to call frequent meetings of the whole membership, or even of the delegates, and as problems have a way of presenting themselves through the year as well as just at convention time, it becomes necessary to designate certain members to do a bit of extra work in our organization. These officers must have been active members of A.S.M.T. and in good standing for at least two years before their names were placed on the ballot to be voted upon by the members. The President, in addition, has spent a year as President-elect, during which time he has kept in close touch with the activities of the organization. He may have had previous years in office also, all of which have served to make him cognizant of the needs of the society and of the development of those needs. The Secretary and the Treasurer may be re-elected to their respective offices. The other six members of the Board, two of whom are selected each year, are elected to serve three years in this capacity. Thus we have an experienced Board of Directors who have the opportunity to delve into all affairs of the society and to serve the membership in that capacity. The Immediate Past President also continues to serve for an extra year and in so doing, contributes his experience for the good of the society. In short, your officers, the Board of Directors, have a tremendous responsibility to carry the load of all interim decisions of the organization and to direct and bring before the House of Delegates all matters in such form that they can be considered carefully by that larger representative body. This does not mean to say that the officers are the only members of the organization that do any work through the year, no indeed! There are, through the year, members of the standing and special committees who do a tremendous job, but that's another chapter.

Except for one committee whose work is demonstrated elsewhere in this issue. We are speaking of the Nominating Committee, made up of six members, two of whom have already served for three years each, two for two years, and two for one year. It has been their responsibility to sift through the names sent in by the various state societies, and judge first who would be most suitable candidates for each of the offices. If the state societies and individual members of the A.S.M.T. have cooperated as requested by the Nominating Committee and have sent in the names of persons from their organizations who have served in office and who are ready to serve the national society as well, their task is not as difficult as it would be otherwise. Their next step is to get the acceptance of those invited to have their names placed on the slate.

So, if every member of A.S.M.T. has fulfilled his own individual responsibility, he will be able to choose those to lead the organization who are best fitted to do so. He has also the privilege of making nominations from the floor of the House of Delegates. Thus it is the personal responsibility of each member of the national society to name (and instruct, if desired by the state organizations) the delegates who are the voting representatives in the annual meeting. No one member of A.S.M.T. is less privileged than any other member to make himself heard nor is less responsible than any other member for the well being of his professional organization. It is wholly a matter of whether or not he accepts his privileges and responsibilities toward his profession and toward his organizations.

For more details on the above statements read your Constitution, Articles IX and X, and the by-laws, Article V, and Article IX, Section 6.

—R. M.

## FROM THE PRESIDENT

Dear A.S.M.T. Members:

I'd like to talk membership to you a little. Some of you are thinking "what else is there to say about it?" And I can understand you so thinking.

The A.S.M.T. membership committee with the splendid cooperation of state membership committees and the Executive Office are doing a remarkably fine job this year. We have nearly 1000 new members since July 1, 1949. This is an excellent and commendable record.

A few days ago I received a copy of the membership figures which will be used to bring our exhibit up to date. Most of our state groups have well under 50 per cent of the ASCP registered medical technologists as members of their state societies! In fact only seven states have over 50 per cent of the ASCP registered medical technologists as state and national society members; and of these only one exceeds 70 per cent. These are discouraging and challenging figures.

If we are to acquire and maintain the prestige befitting our profession, we must interest more who are actively engaged in practicing it. To those of you who will read this letter it is not necessary to point out the advantages of membership in your professional organization. I do urge each of you to make a real effort to impress on that "non-joiner" that the unity of purpose which characterizes our professional organization is what he and every practicing medical technologist wants and needs—the elevation of the status of the medical technologist. This is fundamental, and it lies at the heart of everything we do and of everything we stand for.

Sincerely yours,
IDA L. REILLY

## STATE SOCIETIES

The ARKANSAS S.M.T. will hold its twelfth annual meeting at the University of Arkansas School of Medicine, Little Rock, on April 1, 1950. The CALIFORNIA S.M.T. announces its fourth annual convention on May 6 and 7, at the Hotel Claremont, Berkeley. The IDAHO S.M.T. has sent letters to each physician and registered Medical Technologist in the state giving the address, Box 2616, Boise, as that of their placement service. The ILLINOIS S.M.T. will hold their meeting during the Tri-State Hospital Assembly, May 1 through 3, in Chicago. The annual business meeting will be held on Monday after the program. The KENTUCKY S.M.T. annual session is set for April 29, Phoenix Hotel, Lexington. The first annual meeting of the WASHINGTON S.M.T. was held at the University of Washington School of Medicine, Seattle, this past fall, with a five day Refresher Course sponsored by the State Department of Health and the Washington State Society of Pathologists. The date for the WISCONSIN A.M.T. is April 30, in Milwaukee. The WEST VIRGINIA S.M.T. will meet on April 29 and 30, at the Camden Clark Memorial Hospital, Parkersburg. (See your January journal for dates of other state meetings. The GEORGIA S.M.T. meets on May 4, 5, and 6, 1950. Association of OREGON S.M.T., state convention is May 20. The annual Seminar of the LOUISIANA S.M.T. will be on April 19, 20 and 21, at the Loyola University of the South, New Orleans, Louisiana. The PENNSYLVANIA S.M.T. & L.T. will meet in Pittsburgh on April 15. Oregon State meeting will be on May 20. Your lists of Delegates and Alternates must be sent to 6544 Fannin St. Houston 5, Texas, before May 29 to be certain that your society will be represented by its full quota. Read Article VI of the By-Laws.

## WE CORDIALLY INVITE YOU TO ATTEND THE NATIONAL CONVENTION SHAMROCK HOTEL, HOUSTON, TEXAS

**June 11 through 15, 1950**

That's the formal invite, but off the record, Ah'd like to invite y'all personally. Y'all will miss a whole lot if you don't come. Everything starts with a bang on Sunday evening, and there won't be very many empty minutes until after Thursday night.

Monday afternoon, there's a wonderful trip to San Jacinto Battleground. As Ah said last month, that's the Texas National Shrine. That's where the San Jacinto Monument is, and also the Battleship Texas (that's the ship the Navy used to pull the Missouri off the sand bank).

Tuesday, you'll see "A Bit of Texas." That's all Ah know about it except that it ain't nothing fancy but you'll like it.

On Thursday, there's going to be a fancy dinner. It's going to be in the Emerald Room in the Hotel. There's even going to be a floor show at that shindig.

Now all y'all be sure and come. We won't guarantee the weather but we will guarantee one fine time.

Frances

## PROGRAM COMMITTEE REPORT

### Awards

Any Registered MT (ASCP) is eligible for the Registry Award of $50.00. As the papers competing for this award must be in the hands of the judges by May 1, any not already sent to the Chairman of the Program Committee (any papers also competing for Convention Awards and for the Hillkowitz Memorial must have been in possession of the Chairman since March 15) must be sent to the Chairman by April 15, in time to forward them. Three copies of each paper, typewritten, double-spaced, with any drawings in India ink and suitable for publication, must be submitted. These papers will not be judged by the Convention Awards Committee. If there is still space on the program for any of them at such a late date, they will be read before the convention, otherwise they will be read by title only and will be submitted for publication in the AMERICAN JOURNAL OF MEDICAL TECHNOLOGY.

Papers for the Convention Awards and for the Hillkowitz Award have already been placed on the program and will be submitted for publication.

Papers not read at the convention and not submitted for awards may be sent direct to the JOURNAL for possible publication.

### Program

The complete program will be published in the May issue of the AMERICAN JOURNAL OF MEDICAL TECHNOLOGY. However, in addition to the speakers mentioned in the November JOURNAL, Dr. Asa Chandler, of Rice Institute, Houston, the authority on Hookworms, and the man who wrote "Animal Parasites and Human Disease," will speak on some phase of parasitology. Dr. Charles M. Pomerat, of the University of Texas School of Medicine, Galveston, will give us something on "Tissue Culture Technique and Its Potentials in Clinical Laboratory Work," while some of our own members. Sister M. Antonia, of Louisville, Ky., Jane Maghan, of Minnesota, Nila Maze, of Indiana, Sister M. Dolorosa, of Missouri, Sister Mary James, of Texas, Sister M. Leo Rita, of Missouri, Martha Wilkerson, of Texas (and a brand new member of ASMT), Margaret Krepsky, of Wisconsin, Ruth Guy and Frances Jones, of Texas, to mention just a few, will cover every field of our work from chemistry to

bacteriology and back again. There are another dozen or so speakers already on the program, together with a few of those who failed to send in their titles early enough to make the March JOURNAL deadline. Mr. John Frazer, of the Committee, has a list of the latest movies on various phases of technique. The times of showing will be announced at a later date.

**Workshops**

It would be well if at least one person from each state could attend each of the Workshops so that he could take home to his own society the material gained from the combined experiences of those people from each of the other organizations. For the Finance Workshop there will be an opportunity for the state treasurers or their representatives to get a practical lesson in the routine of dues collection, with several of the experienced treasurers present to make it look easy. The Executive Secretary of ASMT has plans for a kit to be presented to each treasurer, together with a 1, 2, 3 account of "how to do it." Miss Mary Eichman will tell you how to spend your money, how to prepare a budget, and will be there to help explain the national budget, and help you with yours. In the Publicity Workshop, Miss Frieda Claussen and her committee members will give you pointers on how to make your profession known to the general public, and how to do it diplomatically. She will help show you how to avoid some of the pitfalls encountered through over-eagerness and impulsive enthusiasm, and will explain why sometimes it is better to move slowly in order to put an important point "across." In the Education "Problems" Workshop, Dr. J. J. Andujar, Miss Rachel Lehman and Sister Jean Clare will give some practical demonstrations of "how it is done." Then you will have the opportunity to ask your questions and in the ensuing forum period, you will be able to enjoy the opinions of experts.

The Membership representative of each state society will have an opportunity to work with the group which has done so much in investigating the membership possibilities of ASMT during the past year. Led by Mrs. Lucille Wallace, assisted by the Regional Counsellors, or ASMT Membership Committee members, together with Mrs. Ruth Drummond, who will bring in the viewpoint of the Board of Registry, together with an explanation of its facilities for cooperation with ASMT, there's not a state society but will profit by the interchange of ideas. Mrs. Dorothy Foreman, Mrs. Hortense Leach, and Miss Ruth Feucht will help you with more plans for your Recruitment program. Each of them can give pointers from their own experiences, and will be glad to hear yours. Just an hour is allowed for each of the above Workshops, but after a thirty minute recess, there will be another group of five more subjects. Dr. George A. Schenewerk, of San Antonio, Texas, will give some facts about Compulsory Health Insurance. (Did all of you write to your Senators and Representatives about S. 1411 which provides for "government treatment of all mental and physical ills of persons from 5 to 17 years of age and ask them to vote against any bill that will entail more spending by the national government? And H. R. 5940?) Dr. F. J. L. Blasingame will discuss with your state society's representatives some of the phases of "Socialized Medicine" that will affect us. Dr. Lall G. Montgomery and Miss Vernal Johnson will be on hand in still another Workshop to help you with your problems relating to state licensing of Medical Technologists. Finally, for those who want to brush up on their Serological Technique, Mrs. Phyllis Shaw and Miss Dorothy Patras will lead a Workshop on that subject. There will be practical demonstrations and a problem session. With your entertainment reservations, we are requesting that you note in the proper spaces the workshops you expect to be able to attend so that tentative room assignments may be made before the meeting starts.

MISS LUCILE HARRIS, Chairman
Hendrick Memorial Hospital
Abilene, Texas

## REPORT OF THE COMMITTEE ON NOMINATIONS AND ELECTIONS

The following members of the American Society of Medical Technologists will be presented for the vote of the House of Delegates at its annual session on June 14, 1950.

### President Elect (one to be elected)

LAVINA B. WHITE
Pueblo Clinic, Pueblo, Colo.
Member ASMT since 1946
Member publicity ASMT 1947
Advisory Council ASMT 1948-49
Public Relations ASMT 1949-51
Registration ASMT 1949
President Colorado SMT 1948-49
CSMT educational meeting, chairman local arrangements Oct. '47
Paper "Cholesterol and Basal Metabolism" Presented ASMT meeting Denver '47 which received the Sabin award for CSSMT 1947
Vocational Recruitment Committee
CSMT chairman 1949-50
President Pueblo SMT 1948-49

MRS. BEATRICE H. ALLISON
North Tonawanda, New York
Member ASMT since 1940
Delegate from New York on numerous occasions
1940-1943 active in reorganization and chartering of Niagara Frontier Assn.
Charter member and first president New York State Society of Med. Technologists
Active in promoting ASMT in New York
Member Legislative Committee, ASMT at present time
Treasurer Niagara Frontier Soc. 43-44
Member Board of Directors, N. Y. State Society Medical Technologists at present

### Secretary (one to be elected)

SISTER EUGENE MARIE (CARPE) S. C.
Good Samaritan Hospital,
Cincinnati, Ohio
Member ASMT since 1940
Vice President Ohio SMT 1947-48
Member Advisory Committee to Ohio Dept. of Health since 1945
Member Awards Committee ASMT 1949
Recording Secretary ASMT 1949-50
Paper "The Rh Factor in Blood and Related Conditions, presented ASMT 1945. Published AJMT Vol. 12, 1: Jan. '46
Paper "Case Report of Exchange Transfusion in Severe Erythroblastosis" ASMT '47. Published AJMT Vol. 14, 1: Jan. '48
Paper "Recent Developments of the Rh Factor," presented at Ohio State Nurses Ass'n Institute, Jan. 1950
Paper "Densenitization with Rh hapten," Ohio SMT Bulletin, Mar. '50

SISTER M. CLARE (HEATH) O. S. F.
St. Clare's Hospital,
New York, N. Y.
Member ASMT since 1940
Co-organizer of Metropolitan New York Society of Medical Technologists
Vice President Metropolitan Soc. MT. at present time.
Member Board of Directors New York State Soc. Medical Technologists at present.
Member Committee against current licensure bill (state)

### Board of Directors (two to be elected)

ALLYNE LAWLESS
3420 W. Thirtieth Ave., Denver, Colo.
Member ASMT 1934-39
Rejoined ASMT 1944
Charter member Colorado Soc. M.T. (organized 1931)
Recording Secretary CSMT 1931-33
Corresponding Sec. CSMT 1935-37
President CSMT 1937-38
President CSMT 1943-44
Co-chairman ASMT Convention 1947
Member ASMT Constitution and By-Laws Committee 1948-50

MRS. KATHRYN DEEN
U. S. Marine Hospital,
Baltimore, Md.
Member ASMT since 1940 (approx.)
Member Board of Directors Maryland Society of Medical Technologists for two years (1947 and 1948)
President Maryland Society Medical Technologists 1949-50
Delegate to ASMT conventions
Member ASMT Advisory Council 1949-50
Co-Exhibitor ASMT convention 1949

MARY FRANCES JAMES
Medical College of Ala.
Birmingham, Ala.
Member ASMT since 1948
Member ASMT advisory Council 1948-49
Ala. Delegate ASMT Convention 1949
Member Education Committee ASMT 1948-51
Special project: Preparation of Hematology Study Series
Winner 1st prize scientific exhibit 1948 ASMT convention "Unusuall Urinary cellular elements Related to Heavy Metal Therapy"
Chairman Organizational Committee of Alabama State Society of Medical Technologists 1948
President Ala. State Society MT 1948-49
Member Board of Directors Ala. State Society MT 1949-50
Chairman Membership Committee Ala. SMT 1949-50
Paper and Exhibit at spring meeting ASMT 1949

**(No copy received on 4th candidate for this position)**

## VOCATIONAL GUIDANCE AND RECRUITMENT

The Recruitment Chairman, Miss Ruth Feucht, has prepared a letter suitable for sending to college Guidance Counselors, Science Advisors, etc. Mimeographed copies of this are available in small quantities from the Executive Offce. It is suggested especially that they be used as a model and be typed or mimeographed using your own state society letterheads. Reprints of the Vocational Guidance Panel (presented before the A.S.M.T. Convention in June 1949 and printed in the January, 1950, AMERICAN JOURNAL OF MEDICAL TECHNOLOGY) may also be obtained from the Executive Office.

## SEMINAR FUNDS

At the meeting of the Board of Registry at Chicago, October 1950, a sum of $1000 was assigned for seminar funds which may be used by groups of Medical Technologists.

There appears to be a great deal of misconception about the use of the Seminar Fund. The Seminar Fund was designed to provide additional scientific training to Medical Technologists by stimulating the organization of additional scientific meetings. These additional scientific meetings are to be in the nature of short post graduate courses in some phase or phases of medical technology.

The fund was not intended to defray any of the expenses of the annual state meeting. A seminar and the state meeting are not one and the same thing.

The Seminar Fund may be used to pay for the honorarium of the speakers in instances where speakers might ask for payment. It will pay for the travel expenses of a speaker, for the rental of a meeting place if necessary, and for rental of films and projector.

The Fund is not intended to pay for advertising, entertainment, printing of programs and expenditures of like nature.

It is hoped that Medical Technologists will take advantage of this splendid opportunity to further their professional training. Several state groups may form a Seminar.

Application forms will be available for interested groups.

ESTELLE DOWNER
2737 N. Booth Street
Milwaukee 12, Wisconsin

## JOURNAL DEADLINES

Please remember deadline for Journal issues:

for **January,** December 10.
**March,** February 10.
**May,** April 10.
**July,** June 10.
**September,** August 10.
**November,** October 10.

6544 Fannin St.,
Houston 5, Texas.

If we have the dates of your convention in time, we shall be glad to print them. This might serve to notify newcomers to your state as well as some others who might otherwise allow the dates to slip their minds. Perhaps it might be of interest to our readers to have papers presented before these state meetings abstracted for publication in the journal. Be sure that some of those same papers are also presented before our national convention in June.

## SCIENTIFIC EXHIBITS

The Scientific Exhibits Committee for the 1950 Convention is glad to report that space for fifteen exhibits has already been requested. However, there is still space for many more. Let's make this year the best yet and bring outstanding exhibits to share the best in advancement of scientific research with your fellow Technologists. As you who have presented them before know, scientific exhibits are not prepared in a day, so start now and write to one of the Committee, giving details:

Mrs. Olive Polen, 1529 North 5th St., Waco, Texas.
Mrs. Louise Garver, Physicians' Diagnostic Lab., Houston, Texas.
Agatha Taylor, Litchfield, Minnesota.

## ALPHA MU TAU FRATERNITY

Many have asked, who and what is Alpha Mu Tau Fraternity.

Alpha Mu Tau is Greek for first medical technologists. It is a professional fraternity incorporated in the State of Illinois in February, 1948.

Any Medical Technologist in good standing in the A.S.M.T. who has made an outstanding contribution to Medical Technology is eligible for membership.

The object of the Fraternity is the advancement of Medical Technology as a profession. One of the primary means to attain this objective will be the establishment of a scholarship loan fund. This will enable worthy medical technologists who do not have funds immediately available, to pursue higher education in this field.

Meetings are held annually and usually in the place and at the time of the A.S.M.T. annual convention.

Alpha Mu Tau is new. It has great ambitions. It is growing!

Watch for news and announcements of Alpha Mu Tau activities.

## REFRESHER COURSES

The schedule of the Public Health Courses given by the Communicable Disease Center for 1950 is revised as follows: 8.55-8—An additional two-week course in **Laboratory Diagnosis of Tuberculosis** will be given December 4-15, 1950; 8.55-8—The previously announced three-week course in the **Laboratory Diagnosis of Tuberculosis** will be given August 21-September 7, 1950, instead of the dates as announced previously; 8.75-8—An additional one-week course in **Serological Diagnosis of Rickettsial Diseases** will be given November 6-10, 1950.

Information and application forms should be requested from the Chief, Laboratory Services, Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia.

---

A course in **Hematology** will be given at Thorndike Memorial Laboratory, The Boston City Hospital, Boston 18, Massachusetts from June 5 through June 16, 1950. The same course will be repeated from June 19 through June 30, 1950. These courses are designed to offer advanced work in hematology to technologists who are familiar with usual clinical laboratory methods. The tuition fee of $75.00 is payable to Geneva A. Daland. A registration fee of five dollars is required by May 1, 1950. Registration is limited.

## JOURNAL

A limited number of July, September, and November, 1949, issues of THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY are available at a combination cost of $1.50 for the three issues. Single numbers are $0.75 each. Please mail order and check promptly to THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, 6544 Fannin St., Houston 5, Texas.

## CONVENTION ANNOUNCEMENTS

In order that we may know approximately how many persons to expect at each of the entertainment and social events of the Eighteenth Annual Convention of the American Society of Medical Technologists, at the Shamrock Hotel, Houston, Texas, from June 11 through 15, 1950, We are asking that those who plan to attend fill in the blanks on the opposite page and mail to:

Mrs. Vondell S. Knight, Convention Chairman Local Arrangements
920 Peden
Houston 6, Texas.

Keep this page for your own memorandum. ________A.M.

I plan to arrive in Houston on June ________, at ________P.M.

The Registrations and Credentials Desk will open at 8:00 A.M. Sunday, June 11. Be sure to get your Kit at the Information Desk. The hostesses there will be able to direct you to the Church of your choice. A Gray Lines Tour representative will be at the Information Desk on Sunday, with tickets for the Convention Tour of the City of Houston. This tour will cost $1.75. Buses will leave the Shamrock promptly at 2:00 P.M. (There are daily Sightseeing tours at 9:00 A.M. and 2:00 P.M. at a cost of $2.65 if there are less than ten persons in a group. Ten or more will pay $1.75. Except for the Sunday afternoon tour, tickets must be purchased at the Transportation Desk in the Shamrock.)

Advisory Council Meeting at 1:30 P.M. Sunday.

From 6:00 to 8:00 P.M. Sunday, the Texas Society of Medical Technologists will be host to the convention group in the Grecian Room. There we shall meet new friends and greet old ones. (Informal dress.)

Monday, June 12, at 2:00 P.M. buses will leave the Shamrock for the San Jacinto Battle Ground, visit the Battleship Texas, San Jacinto Monument, and a Seafood and Fried Chicken Dinner at the Inn. Transportation, tours, dinner and all will cost $5.30. (Sport clothes and comfortable shoes.)

Tuesday, June 13, at 4:30 P.M., buses will leave the Shamrock for the Marks Ranch, a "Bit o' Texas," Barbecue dinner, Campfire and Cowboy songs and stories, all for $3.75. Go Western—blue jeans and boots, or slacks, or sport clothes will do.

You'll be sorry if you don't plan to take a swim in the Shamrock pool!

Thursday evening, June 15, the high spot of the convention, Banquet in the Emerald Room at the Shamrock at 8:00 P.M. Convention Awards. All the V.I.P. will be there. Dancing afterwards. (Formal, but you'll be just as welcome if you'd rather not.) For $7.50.

If you send a check in advance, please don't ask for a refund after June 1. (We'd rather you don't send any money.)

### HOTEL RESERVATIONS for June 12 through June 15, 1950

At the Hotel Shamrock, Houston, Texas. Make your reservations direct with the hotel.

Rates: Single: $6-$16.

2. Double: or Twin Beds: $8-$18. Single or Double Occupancy.
3. Suite: Room with Boudoir: $10-$20. Single or Double Occupancy.
4. Suite: Parlor and Bedroom: $18-$30. Single or Double Occupancy.
5. Suite: 3 and 4 Room with Boudoir. $30-$40. Single or Double Occupancy. Add $3 for each additional person in 2, 3, 4 or 5.

If a sufficient number of Sisters wish hotel accommodations, a separate block of rooms will be reserved for them. For Sisters' reservations, write to Sister M. Aniceta, St. Joseph's Infirmary, Houston 3, Texas.

If, after your reservation has been made, you find that you will be unable to attend the convention, please be sure to cancel your reservation directly with the hotel.

For reservations outside the hotel, write Miss Shige Nagai, 6608 Plaza Drive, Houston 4, Texas.